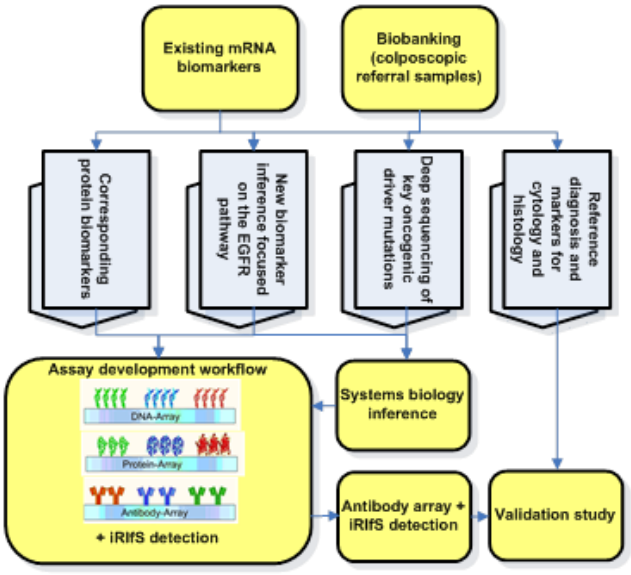

Figure 1. Strategy of the proposal: In a first step, existing biomarkers, new biomarkers generated through systems biology exploration and deep sequencing of patient samples (CellCall) together with existing data from repositories allows derivation of potential protein biomarkers. DNA targets (expression vectors of genes) are displayed as an array and “copied” via cell free synthesis into protein arrays (ALU-FR). By panning of an antibody-phage library (CellCall & ALU-FR) onto this array (against each of the proteins), we planned to develop antibodies, which would then be characterized against all proteins in terms of specificity, sensitivity and cross-reactivity with a label-free detection system (Biametrics). In a second step, the evaluated antibodies could be displayed as an array (ALU-FR) and lysates of clinically characterized patient samples (CellCall & TCD) tested for binding, again with the same label-free system (Biametrics). As a final evaluation, the new antibody arrays were to be compared to state of the art gold standard histology (CellCall & TCD).

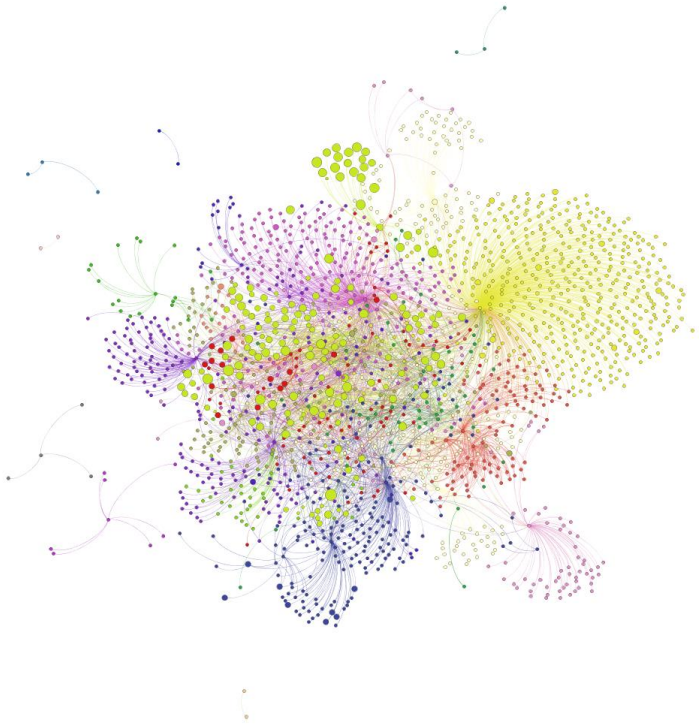

Figure 2. Gephi visualisation of the GDS3233 dataset form GEO database after SIRENE inference and the identification of communities. Communities are in different colours and the spot sizes are corresponding with expression of the given gene

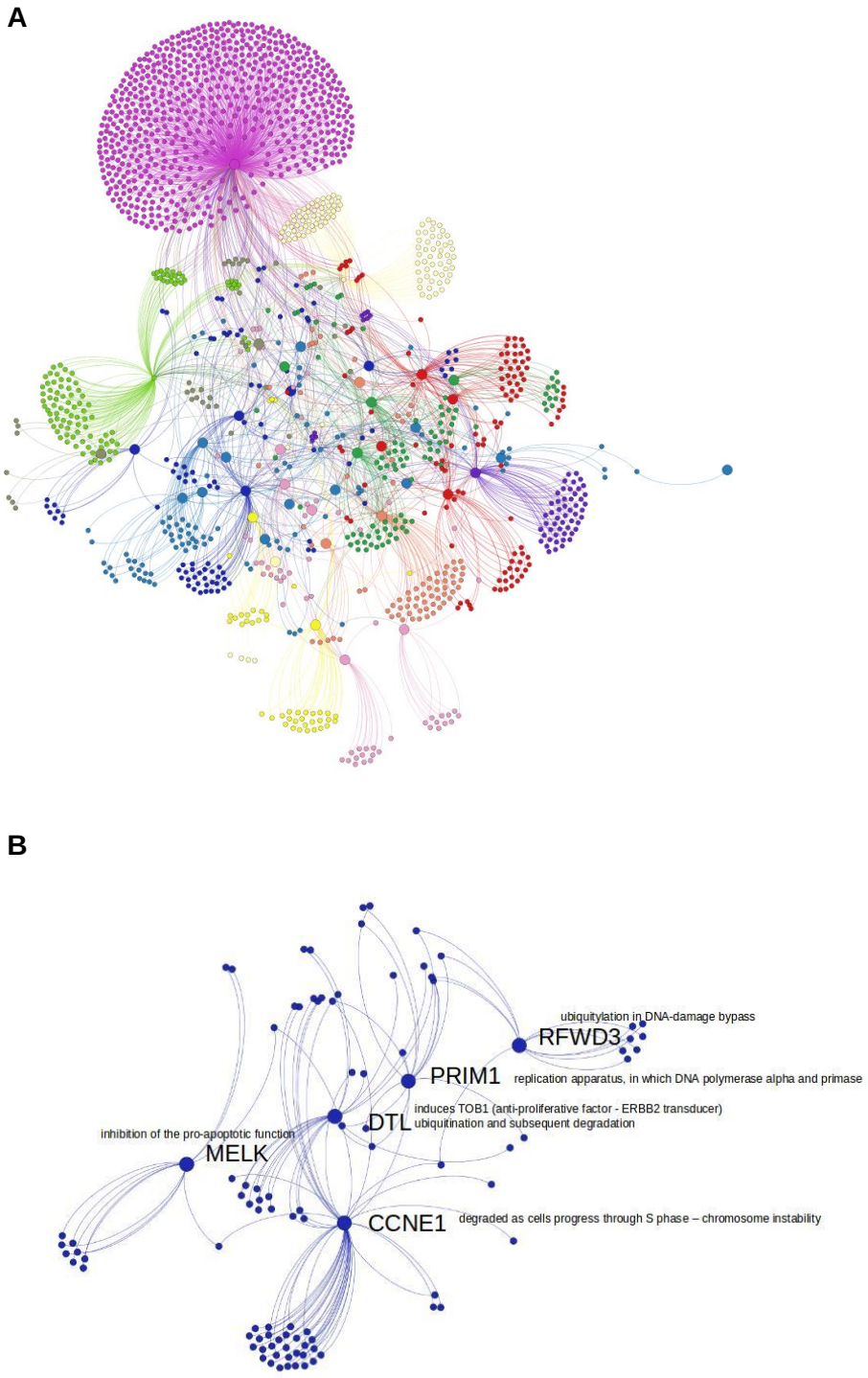

Figure 3: **(A)** Interactome map of Community 16. New communities were calculated on the basis of interactome data retrieved form PINA v2.0. **(B)** Community 6 of the Community 16 interactome map.
Colours representing the different communities and the size of the gene symbols are proportional to their over expression in cervical cancer.

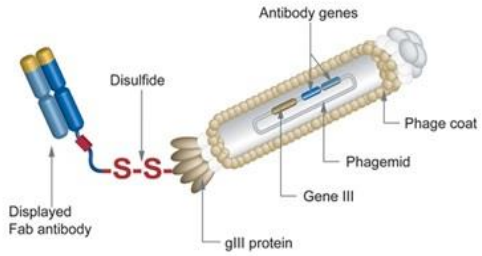

Figure 4: CysDisplay technology

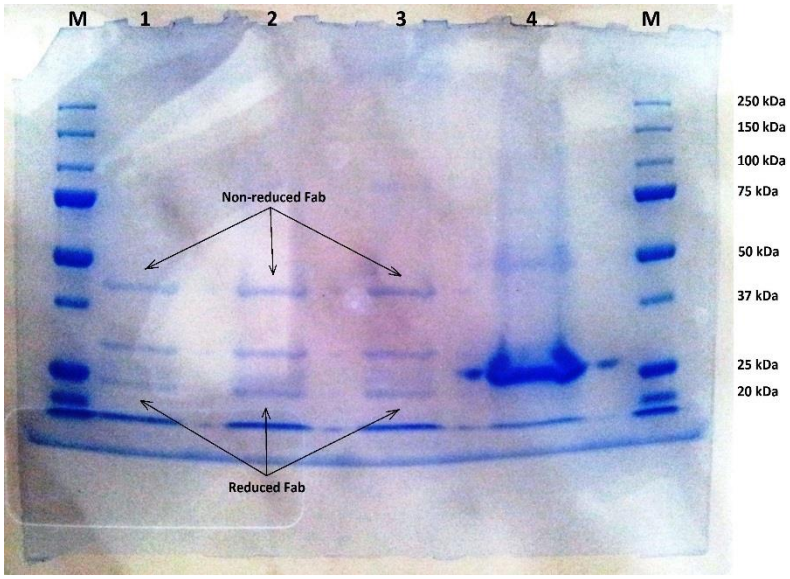

Figure 5: Reducing SDS-PAGE of the in vivo expressed phage-derived Fab antibodies for DSG3

Lanes 1-2-3: Three clones contain VHVλ genes in pMORPHx11_Fab_FH expression vector. Lane 4: Positive control green fluorescent protein (GFP; 27 kDa) M: Precision Plus Protein Standards (Bio-Rad)

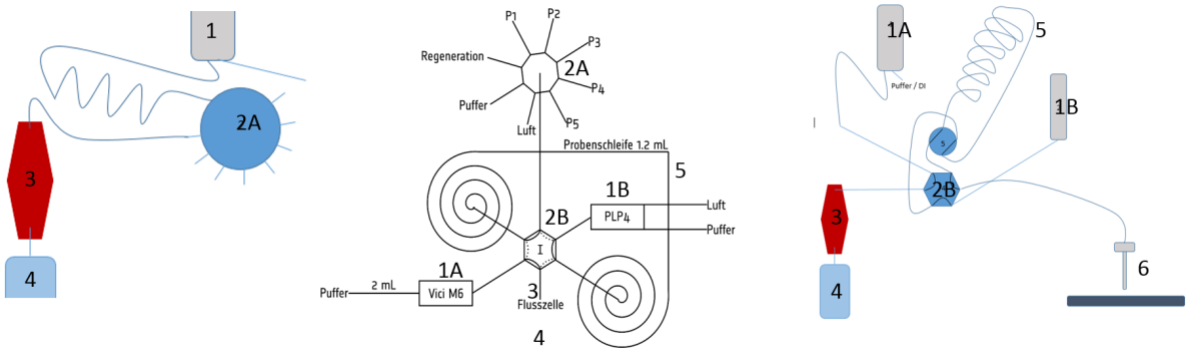

Figure 6: Scheme of the different functional units of the SYSTEMCERV demonstrator device

Based on the prototype (left) and the current setup at Biametrics (middle) we designed a combined demonstrator layout (right). The initial setup (left) contained a pump (1), 1 9-way vent (2A) to take in or pump out samples, a flow cell (3) in which the binding and the iRIf-detection takes place and a waste (4). Biametrics setup (middle) contains similar modules, but yields also a sample loop (5) and two pumps (1A and B). One is providing constant flow conditions like in a Biacore and the other is used for loading the sample loop. To realize this a second vent (2B) with 6-ways is needed. Both layouts are merged for the SystemCerv demonstrator (right), which now allows to run in a Biacore-like mode by a driving pump (1A), providing continuous flow, whilst a loading pump (1B) loads the sample loop.

Fig 7(A)

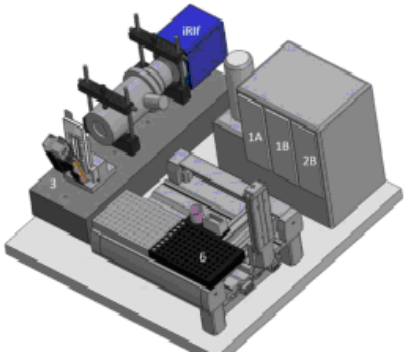

Fig 7(B)

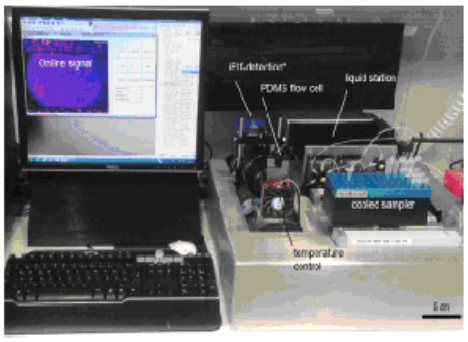

Figure 7: (A) Final design of the device; (B) Final SYSTEMCERV demonstrator device. The device is now fully automated to run autonomous a complete assay protocol. The user only has to load the samples into the rack and to put in the flow cell and the microarray. The manual assembly of flow cell and microarray is still a skilful challenge and needs experience and training. As the flow cell is 40 µm in height a dust free environment like a sterile hood or a clean bench is recommended.

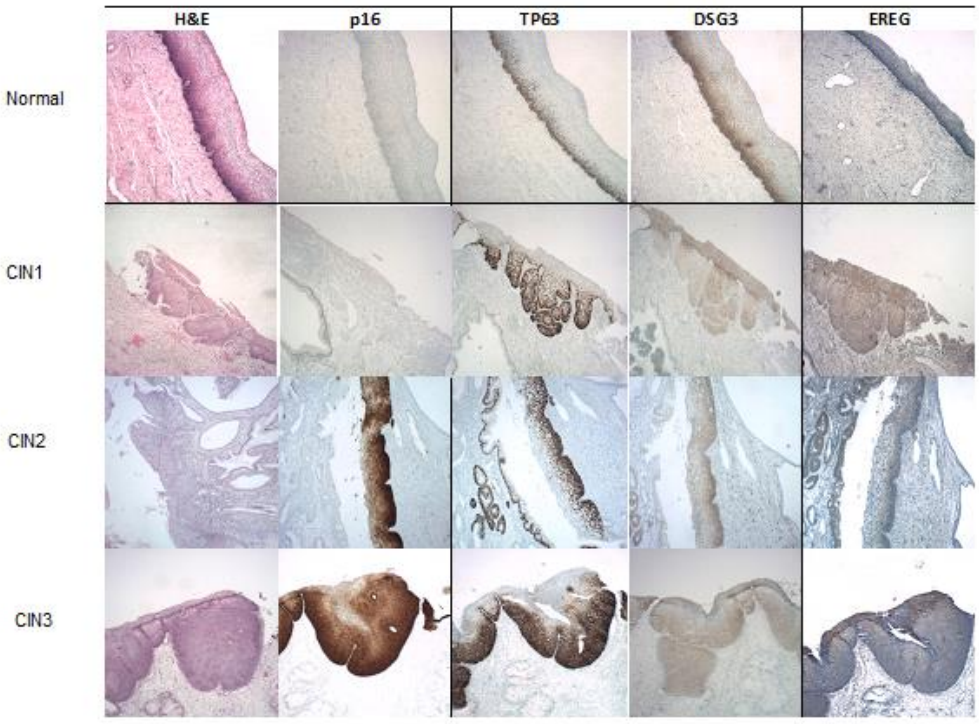

Figure 8: Immunohistochemistry of cervical squamous epithelium from Normal, CIN 1, CIN 2 and CIN 3 for p16, TP63, DSG3 and EREG (magnification 5x)

Table 1: Input and output phage titres defined during the panning rounds for DSG3

Sub-library	*Input 1st round*	*Output 1st round*	*Input 2nd round*	*Output 2nd round*	*Input 3rd round*	*Output 3rd round*
VHVκ phage pool	~10^{13}	$1{,}3\times10^{7}$	failed	$1{,}6\times10^{5}$	$5{,}2\times10^{11}$	$2{,}1\times10^{5}$
VHVλ phage pool	~10^{13}	$3{,}5\times10^{6}$	failed	$1{,}3\times10^{5}$	$4{,}4\times10^{11}$	$3{,}4\times10^{4}$

Table 2: Input and output phage titres determined during the panning rounds for RTKN2

Sub-library	*Input 1st round*	*Output 1st round*	*Input 2nd round*	*Output 2nd round*	*Input 3rd round*	*Output 3rd round*
VHVκ phage pool	~10^{13}	$3{,}9\times10^{5}$	$1{,}7\times10^{12}$	$4{,}1\times10^{4}$	$6{,}7\times10^{11}$	$4{,}9\times10^{4}$
VHVλ phage pool	~10^{13}	$1{,}9\times10^{5}$	$1{,}5\times10^{12}$	$3{,}3\times10^{4}$	$4{,}4\times10^{11}$	$1{,}6\times10^{4}$