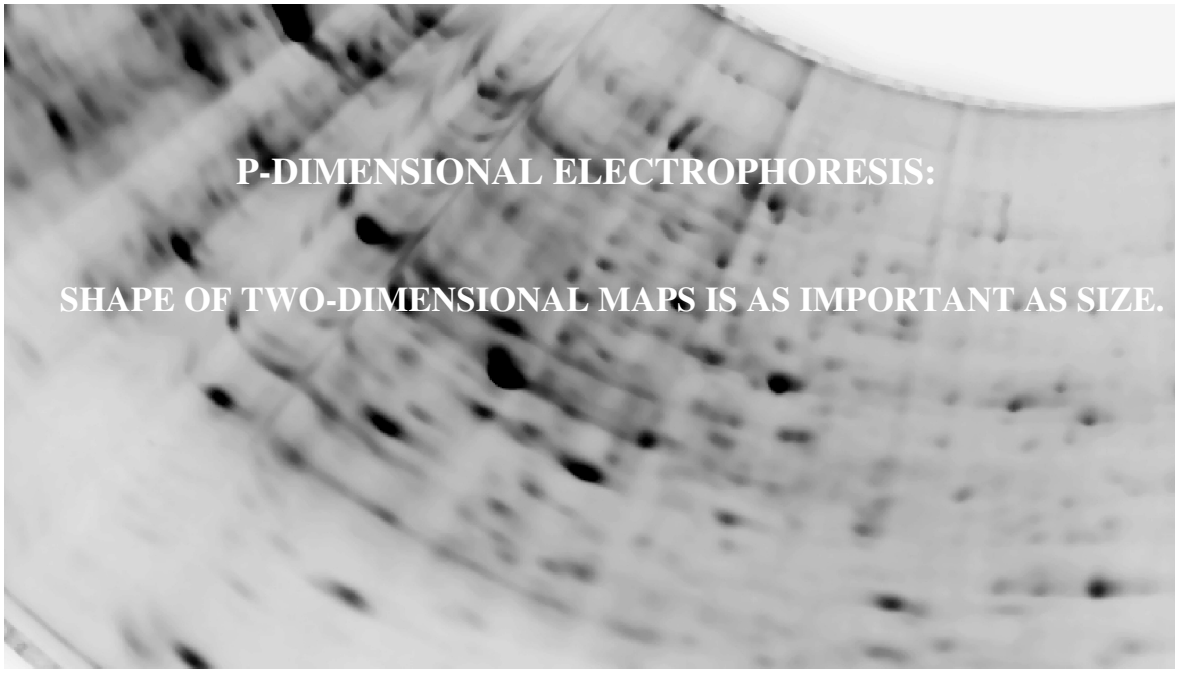
P Dimensional



State of the art:
Two-dimensional electrophoresis (2-DE) consists of a tandem pair of electrophoretic separations: in the 1st (IEF), proteins are resolved in according to their isoelectric points (pI) using immobilized pH gradient (IPG) electrophoresis, and in the 2nd dimension, proteins are separated according to their approximate molecular weight (Mr) using sodium dodecyl sulfate poly-acrylamide-electrophoresis (SDS-PAGE).
2-DE is currently one of the best techniques that can be routinely applied for parallel quantitative expression profiling of complex mixtures of proteins, such as cell lysates and biological fluids. Furthermore, 2-DE produces maps of proteins where changes in protein expression, isoforms and post-translational modifications are assessable at a visual level.
However, this technique is criticized as being low-throughput, in part due to the time-consuming process of image analysis that is necessary to determine differential protein expression. This process can be laborious due to gel-to-gel variations that confound the analysis process. Moreover, another problem is the scarce resolution achieved when working with complex biological samples, especially when extended gradients are used instead of narrow (3 pH unit wide) or ultranarrow (down to 1 pH unit wide) IPG-strip. This in turn leads to the phenomenon of "spot overlapping", by which proteins with similar features (pI and Mr) are not resolved either in the first dimension or in the second one, thus merging in a unique spot in the final map [1].

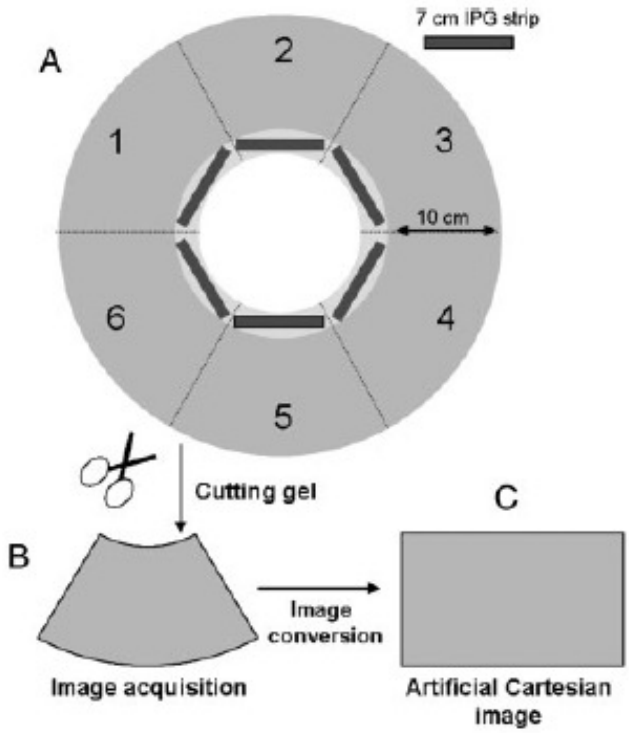
The innovation: The P-Dimensional electrophoresis (2-PE) has the aim to improve the resolution of proteins with close pI and Mr values and the map reproducibility, as compared to the traditional 2-DE technology.
2-PE (patent pending) is still based on the coupling of IEF in the first dimension and SDS-PAGE in the second dimension, but it takes advantage of a SDS-PAGE step with a radial electric field instead of a parallel one (Figure 1)

Here we point out differences and advantages of 2-PE in comparison to the traditional method 2-DE:

INCREASED RESOLUTION AND REPRODUCIBILITY

- The “second dimension” is carried out in an innovative apparatus, where the lines of force of the electric field are determined by circular and concentric electrodes. Since spots with close but not equal pI and Mr are moved by diverging lines of forces, their resolution increases during the radial separation, by a factor proportional to the migration distance. At the end of this document, we report some figures of laboratory results as explicative examples to show in 2-PE the increase in the resolution of protein spots with equal Mr but different pI with respect to 2-DE. All the accessories required to perform the second dimension are reported in instrumentation’s manuals, for a more comprehensive explanation.

- The loading of two or more (depending on their length) IPG strips on a single SDS-PAGE gel greatly improves the reproducibility with respect to 2-DE and makes the subsequent analysis faster and more reliable, due to easier spot matching [2]. In this regard 2-PE, thanks to the particular shape of the gels, makes it possible to run a number of IPG strips at the same time, twice as many as the traditional method. A possible IPG loading scheme in 2-PE is reported in the following figure.



Schematic workflow for multi-strip IPG loading on one radial gel. Panel A: loading six 7-cm-long IPGs, each occupying 1/6 of the internal circumference, into P-Dimensional electrophoresis (2-PE). Panel B: each of the six sectors is cut and acquired as digitalized image. Panel C: each image is converted from polar to Cartesian coordinates and analyzed by Delta2D.

MORE TIME- AND COST-EFFICIENT:

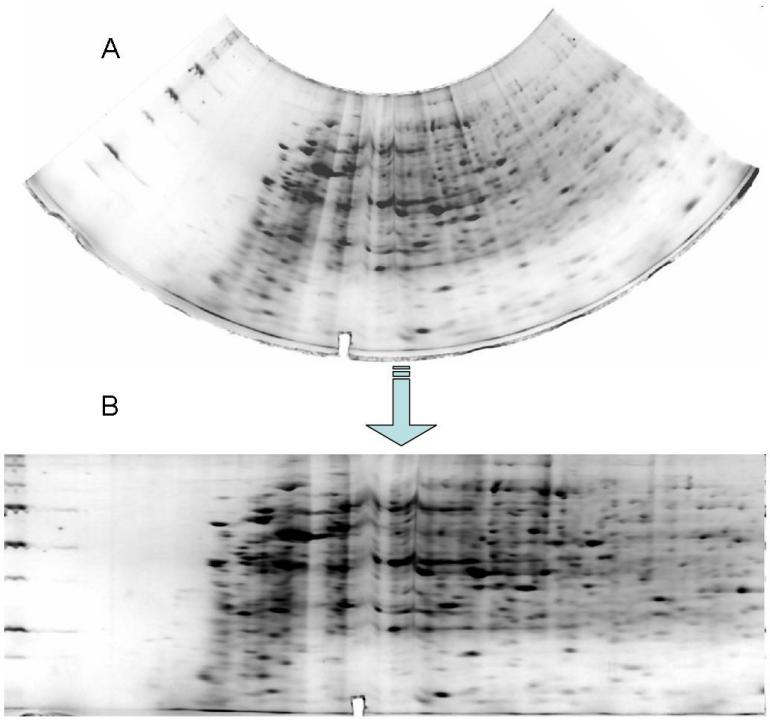
- With the higher resolution, the spot picking up, a delicate and usually manually work, is simplified.

- Both of the electrophoretic devices used to carry out the 2-PE are adaptable to strips and gels of different sizes. Hence, with one system, the laboratory can work with gels of different sizes (depending on its needs) while it often happens that electrophoretic systems are designed to accommodate only gels of one specific size.

-The multi-strip loading configuration would also allow for reduced consumption of chemicals (such as the electrophoretic buffer, polyacrylamide and staining/destaining solutions) and the time required for an experiment.

- Generally each 2-DE gel requires from three to four hours of graphics processing work for the qualitative and quantitative analyses. The best quality image you can get through the 2-PE (where spots appear more spread out) will also significantly reduce the time for this final step. Furthermore, the ability to load more strips simultaneously on the same gel allows for a faster and easier comparison of different electrophoretic runs since these are conducted in identical conditions.

- Thanks to the collaboration of Decodon GmbH, we have developed a program which allows for the conversion of radial dimensional images into cartesian ones, a useful step since the Cartesian visualization is the more user-friendly data view for image elaboration. The following figure shows that, thanks to this program, it is possible to obtain perfect cartesian maps (B) from the radial ones (A), retaining the increased resolution.



-The best quality of gel can reduce the number of technical replicates needed for the statistical analysis.

In conclusion there are many advantages coming from this innovation, especially in terms of resolution, timing and cost for experiment. As the natural evolution of 2-DE, P-Dimensional electrophoresis has all the requisites to become the new gold standard for the electrophoretic analysis of protein expression patterns in cells, tissues and other biological samples.

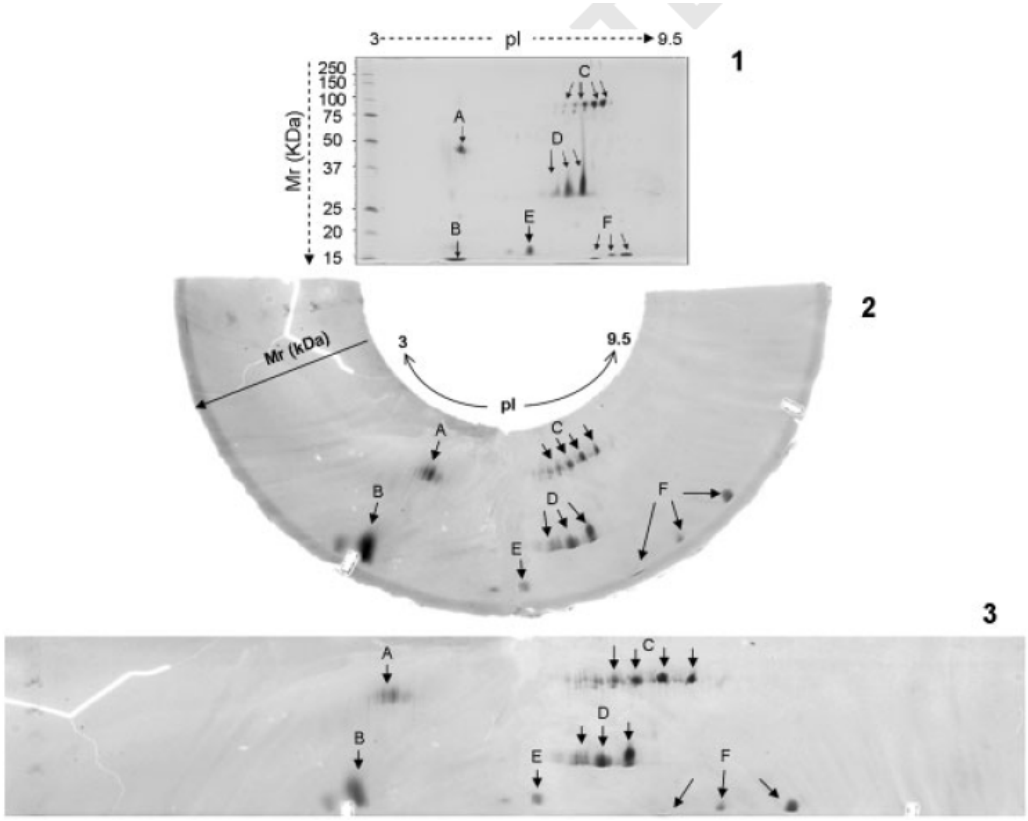
References:

[1] Proteomics. 2005, 5: 2385-95. Spot overlapping in two-dimensional maps: a serious problem ignored for much too long. Campostrini N, Areces LB, Rappsilber J, Pietrogrande MC, Dondi F, Pastorino F, Ponzoni M, Righetti PG.
[2] Acta biochimica et biophysica sinica 2003, 35(7): 611-618. Multi-strips on one gel method to improve the reproducibility, resolution power and high-throughput of two-dimensional electrophoresis. YUAN Quan, AN Jie, LIU Ding-Gan, ZHAO Fu-Kun.

Experimental evidences:

TEST ON STANDARD PROTEIN MIX:

Protein	pI	Mr (kDA)
Ovalbumin (A)	4,7	45
Alfa-lactalbumin (B)	4,5	14
Conalbumin (C)	6	76
	6,3	76
	6,6	76
Carbonic anhydrase (D)	6	31
Hemoglobin (E)	7.0	64
Myoglobin (F)	7,3	17,6
	6,8	17,6



Traditional (panel 1) and radial (panel 2) 2-D maps of a standard protein mixture. Panel 3 represents the radial image after the conversion of coordinates from polar to Cartesian.

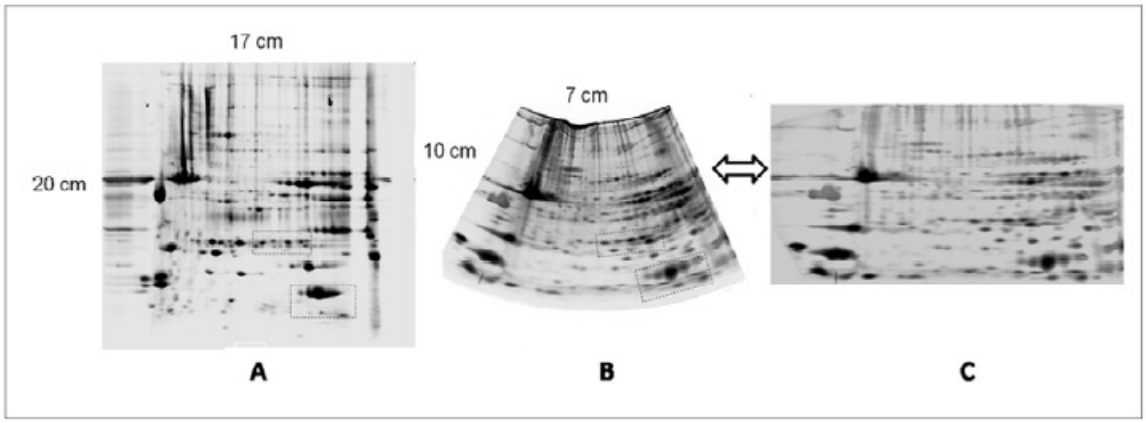
TEST ON COMPLEX PROTEOMES WITH SHORT IPG STRIPS AND THE "MULTI STRIP ON ONE GEL METHOD":

To investigate the comparison of 2-PE vs. 2-DE, we report a qualitative evaluation of results obtained with the different approaches, but using Cartesian gels with an area about twice that of radial gels.

Experimental design

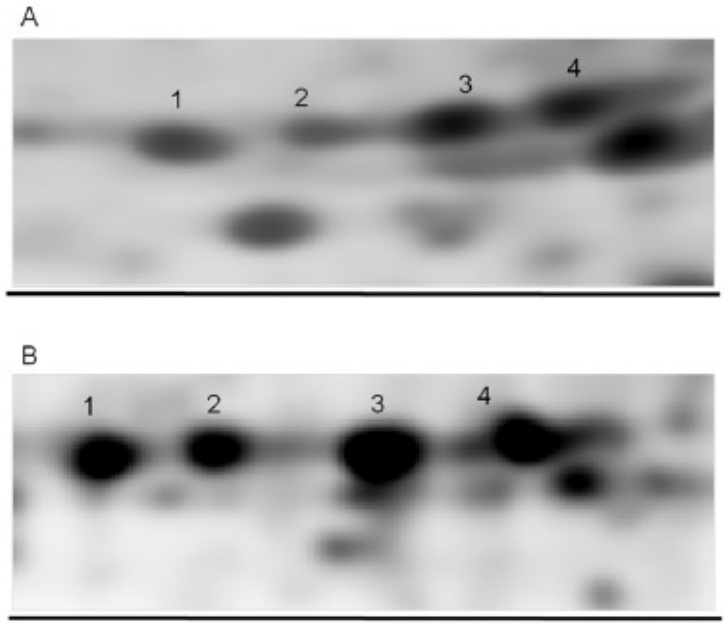
	2-DE	**2-PE**
1st dimension lenght (cm)	17	7
2nd dimension lenght (cm)	20	10
Gel area	340	130
Sample loading per map (mg)	1.2	0.12
Porosity in 2nd dimension (%)	8-18 (gradient)	12
N° of maps per gel	1	6
N° of technical replicates	23	23

RESULTS

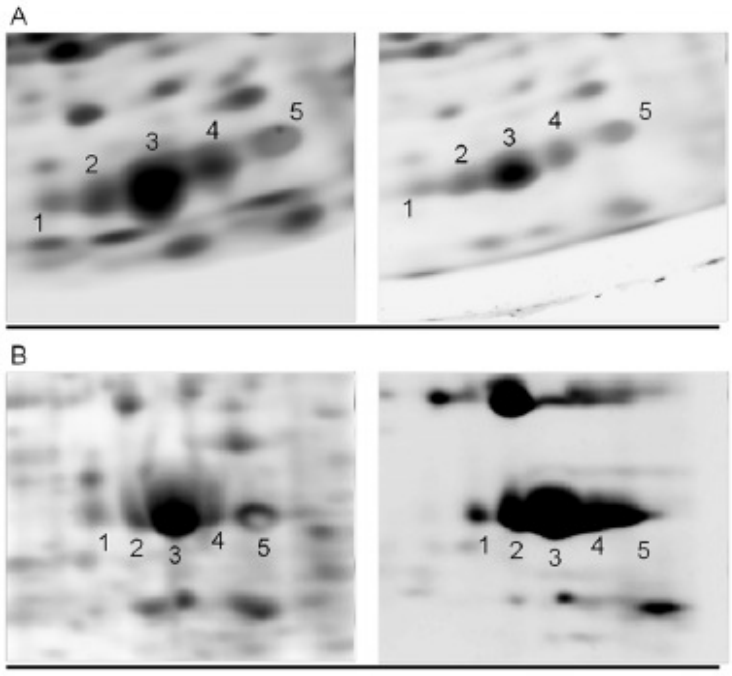


Representative gel images obtained with 2-DE (panel A) and 2-PE (panel B). Panel C shows the radial map after the Delta2D assisted conversion of coordinates from polar to Cartesian. The lengths of the axes are reported. The highlighted areas of the gels are those reported in Figure 2 and 3 as enlarged sections. Sample load: 1.2 mg of total protein in A vs. 120 mg in B. Protein samples were obtained from Longissimus dorsi (LD) muscle

Thanks to the increased protein loading (~30x) in the Cartesian set, we observed the appearance of some spots (+10%), that were completely absent in the radial set. Due to the possibility of running up to six IPG strips in the same radial gel, the radial set showed an increased reproducibility (+20% efficiency of matching).
Hence, comparative analysis reveals that, despite the smaller area, the absence of a gradient of porosity in the 2nd dimension and the reduced loading, the radial set show a performance similar to the Cartesian one. As an extra bonus, strings of spots are more resolved in the radial gel format, especially in the 10 to 30 kDa region, where the gel area fans out leaving extra space for spot resolution (see the following figures).



Enlarged sections of gel images obtained with 2-DE (panel A) and 2-PE (panel B). The similar resolution of spots 1–4 in the two experimental sets can be visually appreciated. Protein spots 1–4 of 2-PE and 2-DE maps were identified by mass spectrometry analysis as different isoforms of triosephosphate isomerase.

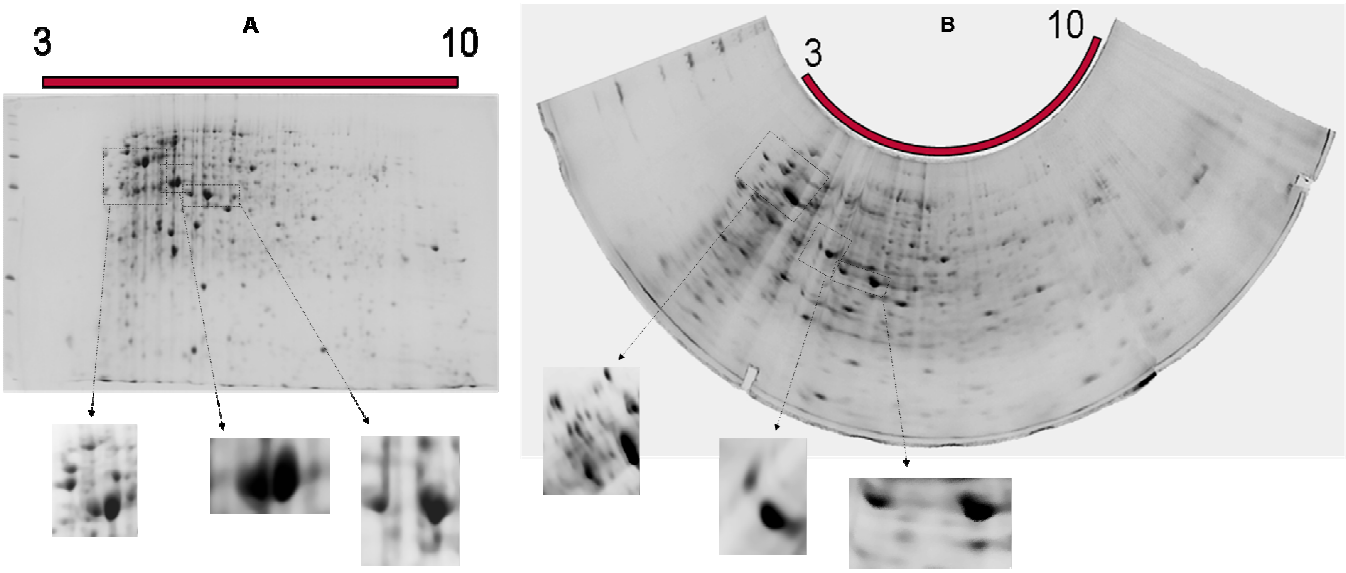


Enlarged sections of gel images obtained with 2-DE (panel A) and 2-PE (panel B). The better resolution of spots 1–5 in the radial gel format can be visually appreciated. Protein spots 1–4 of 2-PE and 2-DE maps were identified by mass spectrometry analysis as different isoforms of myoglobin

Conclusions

The comparative analysis reveals that, despite the smaller area, the absence of a gradient of porosity in the second dimension and the reduced loading, the radial set show a performance similar to the Cartesian one, but with the advantage of an associated increased reproducibility.

TEST ON COMPLEX PROTEOMES WITH LONG IPG STRIPS AND THE "MULTI STRIP ON ONE GEL METHOD":



Traditional (panel A) and "circular" (panel B) 2-D maps of cytoplasm water-soluble proteins from *S. maltophilia*. IPGs: 18-cm long non-linear 3-10 pH gradient. SDS-PAGE: 12 %T Tris-Glycine gels.

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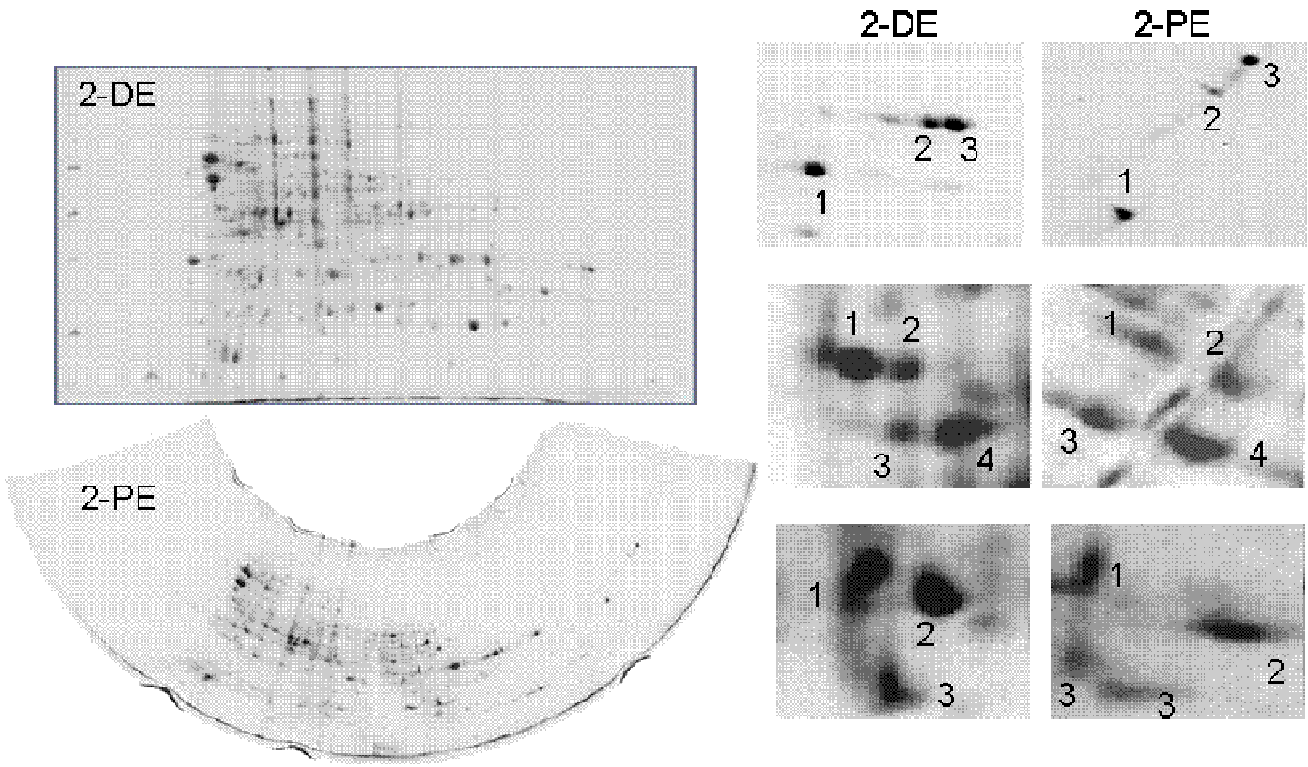
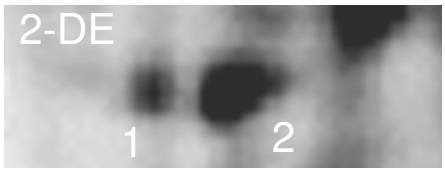
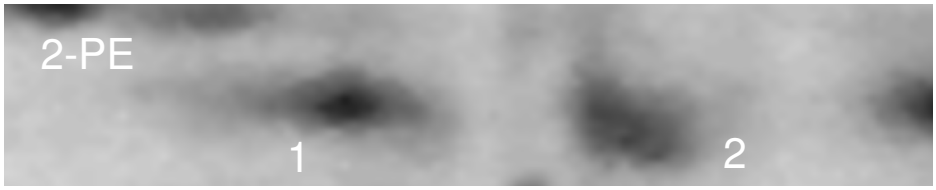


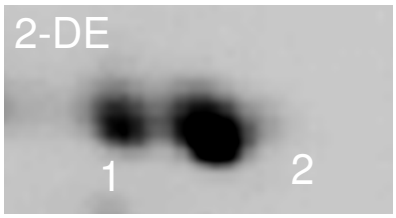
Traditional (panel A) and "circular" (panel B) 2-D maps of cytoplasm water-soluble proteins from *E. Coli*. IPGs: 18-cm long non-linear 3-10 pH gradient. SDS-PAGE: 12 %T Tris-Glycine gels.

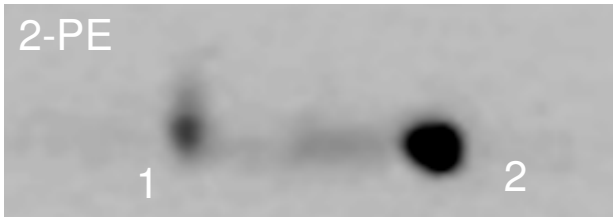
ENLARGED ZOOM VIEWS

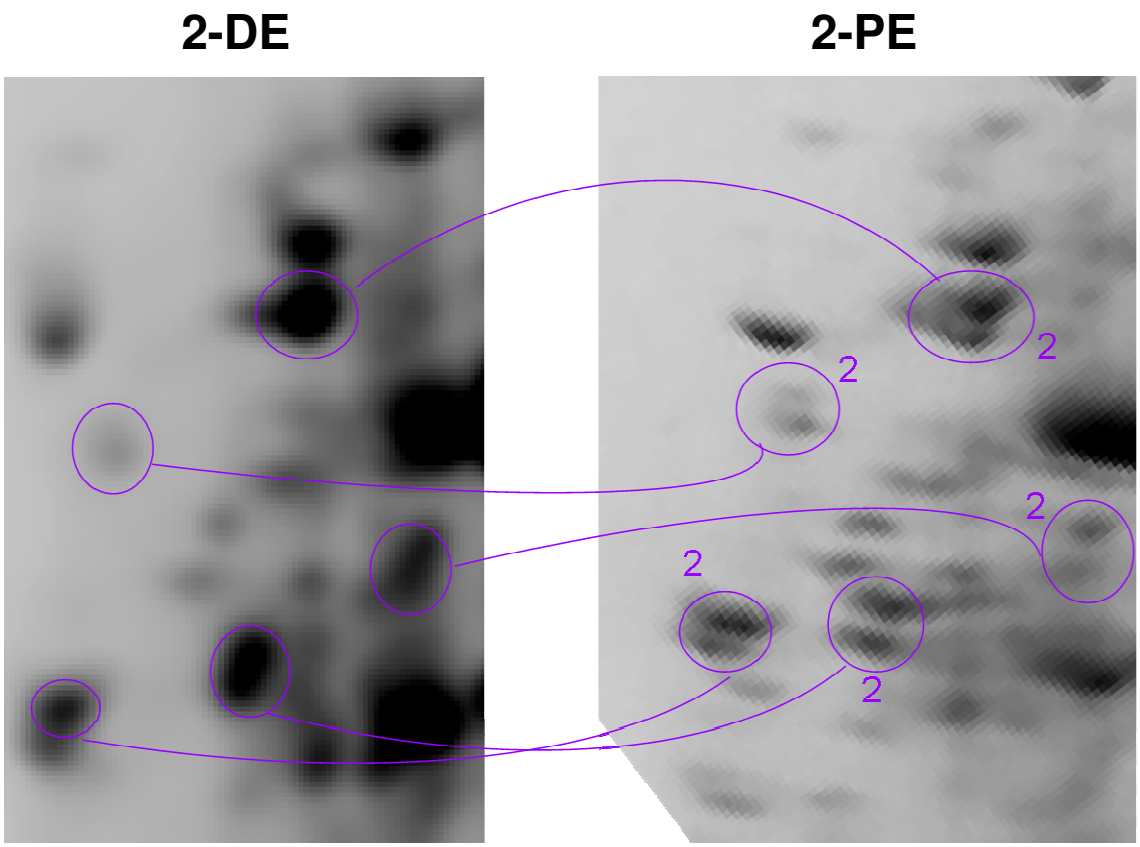
Enlarged zoom views, to appreciated the higher resolution on 2-PE maps respect to 2-DE.











An **interesting side effect** of the radial electric field geometry: in association with the distancing of spots during the electrophoretic migration, we observed also a flattening of the spots, which increases the resolution along the y-axis of the 2-D map.

P-DIMENSIONAL ELECTROPHORESIS RELATED PUBLICATIONS

Type of publication: Patent pubblication
Inventors: Renato Millioni, Antonio Guadagnino, Manuela Miuzzo, Elisa Barina, Tommaso Salata, Piergiorgio Righetti

Method and apparatus for the simultaneous separation of biological molecules by two dimensional electrophoresis.
Pub N° WO/2006/056861, International App N°:PCT/IB2 005/003519

App numb	Country	Status
2820/CHENP/2007	India	Published
10-2007-7011797	Korea	Pending
2007-542164	Giappone	Published
200580040550.5	Cina	Published
05850670.0	EP	Published
2,589,232	Canada	Published
11/791,728	USA	Pending

Type of publication: peer reviewed pubblications

- Millioni R, Polati R, Menini M, Puricelli L, Miuzzo M, Tessari P, Novelli E, Righetti PG, Cecconi D. Polar electrophoresis: shape of two-dimensional maps is as important as size. PLoS One. 2012;7(1):e30911. Epub 2012 Jan 23.

- Millioni R, Miuzzo M, Puricelli L, Iori E, Sbrignadello S, Dosselli R, Cecconi D, Tessari P, Righetti PG. Improved instrumentation for large-size two-dimensional protein maps. Electrophoresis. 2010 Dec;31(23-24):3863-6.

- Millioni R, Miuzzo M, Antonioli P, Sbrignadello S, Iori E, Dosselli R, Puricelli L, Kolbe M, Tessari P, Righetti PG. SDS-PAGE and two-dimensional maps in a radial gel format. Electrophoresis. 2010 Jan;31(3):465-70.

Type of publication: Meeting Abstracts

- "Proteomics & Pathology" (Valencia, 10-14/02/2007), Joint Congress of the Spanish Proteomics Society and the European Proteomics Association

P-Dimensional electrophoresis: the vision of proteome at 360 degrees
Renato Millioni, Manuela Miuzzo, Paolo Antonioli, Martha Elena Mendieta, Antonio Guadagnino, Paolo Tessari, Pier Giorgio Righetti

- "7th International Symposium "Amino Acid/Protein Metabolism in Health and Disease: Mechanisms and Pathways Controlling Protein Expression and Turnover" Padova, Italy –4-5/06/2008

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