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# Gel Electrophoresis as Quality Control Method of the Radiolabeled Monoclonal Antibodies

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## 1. Introduction

Neurodegeneration is the leading term for the progressive loss of the neuron structure, including death of neurons. Many neurodegenerative diseases including the specific diseases - such as Parkinson's, Alzheimer's, and Huntington's occur as a result of the neurodegenerative processes. As research progresses, many similarities appear which relate these diseases to one another on a sub-cellular level. Discovering these similarities offers hope for therapeutic advances that could ameliorate many diseases simultaneously. There are many parallels between different neurodegenerative disorders including atypical protein assemblies as well as induced cell death followed by an apoptosis. Apoptosis is a form of the programmed cell death in the multicellular organisms. It is one of the main types of the programmed cell death, and, last but not least, involves a series of the biochemical reactions leading to a characteristic cell morphology changes, and, finally, death. In according to the previously mentioned knowledge, there is a necessity to develop an imaging method which describes these cellular changes. The principal goal of the investigation monoclonal antibodies and their fragments is to examine the possibility of developing of an imaging radiotracer that would be specific for cytoskeleton of destructed dendrites and neuronal bodies. One of the suitable fitting marker, specific for neuronal tissue, performs anti III  $\beta$ -tubulin (bTcIII) antibody - TU-20 with molecular weight 150 kDa and its scFv fragment with molecular weight 27.7 kDa. The scFv fragment of TU-20 was synthesized for its higher mobility through tissue and vascular barriers. Biochemical characteristics (especially immunoaffinity) of the specific binding substance - anti III  $\beta$ -tubulin scFv fragment - is preserved, and, moreover, the biological availability is much better than in case of the whole antibody. See the structure in the Fig. 1.

To examine this hypothesis, it is necessary to radiolabel both substances with  $^{125}\text{I}$  and  $^{123}\text{I}$ . The next step is chemical analysis and, furthermore, biochemical properties are extensively investigated. The quality control, performed by gel filtration, electrophoresis, ELISA testing determines adequate properties of the radiolabeled substances for further studies.

Affinity coupling and RIA analytic methods occur under development with focusing on specifics of the antibody and its fragment behavior. *In vitro* experiment shows an extent of the preserved binding specificity of the species by incubation of the both radiolabeled substances with mice brain slices followed by an autoradiography.

The *in vivo* biodistribution confirms behavior of elimination of the radiolabeled TU-20 and scFv from mice. The bi-exponential model for two-phase clearance to determine short phase half-life  $t_{1/2\alpha}$  and long phase half-life  $t_{1/2\beta}$  values is used. For comparative study, a transgene population G93A1 Gur was chosen to show different behavior of the substances in normal mouse and in modified organism with amyotrophic lateral sclerosis (ALS).

The main objective of this work is to develop a method for direct imaging of the structural degradation of peripheral neurones by various types of neuropathies.

## 2. Methods and materials

The monoclonal antibody TU-20 and its scFv was purchased from Exbio, CZ. The antibody recognizes the peptide sequence ESESQGPK. ScFv TU-20 is a recombinant protein expressed in *E. coli*. (Dráberová et al., 1998)

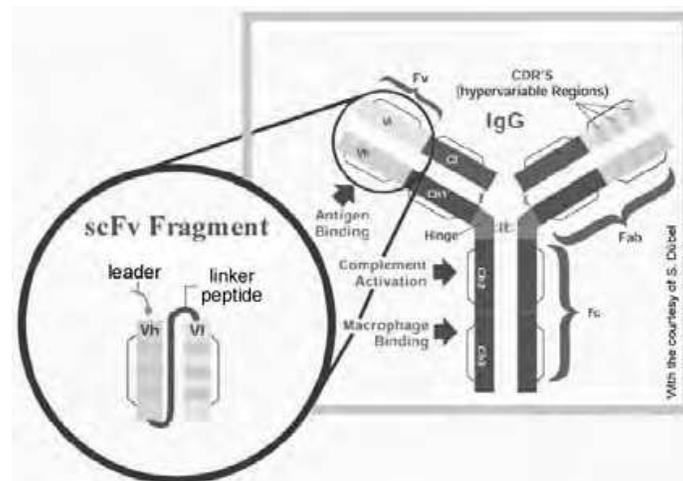


Fig. 1. The structure of the monoclonal antibody on the base of IgG and its scFv fragment.

### 2.1 Radioiodination of the antibody

$^{125}\text{I}$  ( $T_{1/2} = 59,4$  h) radioiodination of TU-20 and scFv TU-20 was performed via chloramine-T with or without stopping reaction with sodium thiosulfate agent. The ratio of an amount of TU-20 to radioactivity was  $1\ \mu\text{g}$  to 5.5-7.0 MBq of  $^{125}\text{I}$ . The ratio of an amount of the fragment to radioactivity was  $1\ \mu\text{g}$  to 1.5-2.0 MBq of  $^{125}\text{I}$ .  $^{123}\text{I}$  ( $T_{1/2} = 13,3$  h) radioiodination of the fragment scFv TU-20 was performed via chloramine-T with stopping reaction with sodium thiosulfate. The ratio of an amount of the fragment to radioactivity was  $1\ \mu\text{g}$  to 3-5 MBq  $^{123}\text{I}$  (Švecová et al., 2008). The structure of the radiolabeled antibody is shown in the Fig. 2.

The monoclonal antibody TU-20 was radioiodinated by using either chloramine-T or iodogen as an oxidizing agent. Iodination via chloramine-T was provided in two alternative ways: either with or without stopping a reaction by a reducing agent (Dráberová et al., 1998).

The reaction was performed under following conditions:  $10\ \mu\text{l}$  of TU-20 ( $1\ \text{mg}/\text{ml}$ ) was transferred to  $10\ \mu\text{l}$  phosphate buffer (PBS,  $0,01\ \text{M}$ ,  $\text{pH}\ 7,4$ ) in a reaction vessel and  $^{125}\text{I}$  radioactivity (approximately 5,36 MBq) was added. Finally, the solution of chloramine-T in PBS ( $0,1\ \text{mg}/\text{ml}$ ) was added to the reaction vessel. The amount of chloramine-T ranged from  $0,5$  to  $6\ \mu\text{g}$  per  $10\ \mu\text{g}$  of the antibody. After the reaction time (60 seconds), during which the

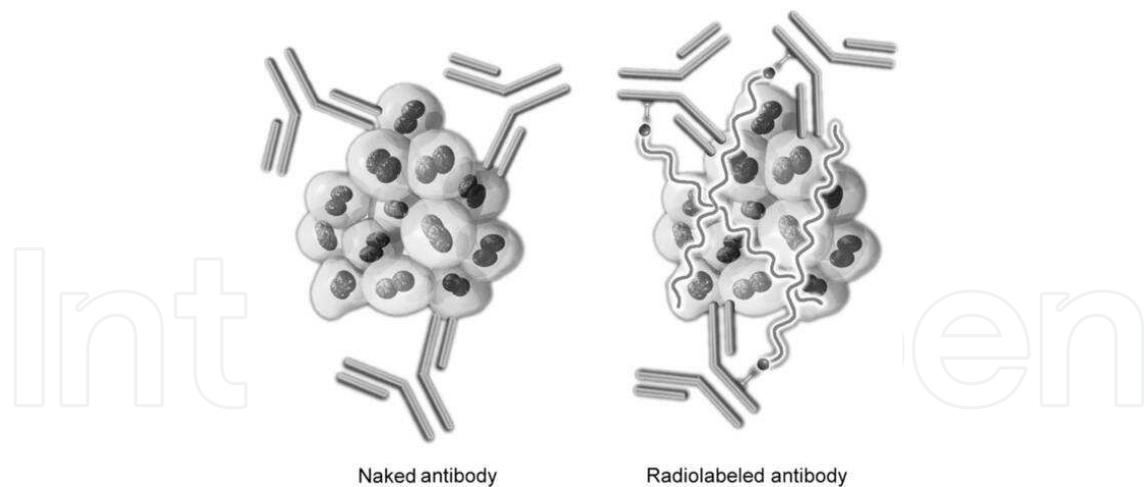


Fig. 2. Radiolabeled monoclonal antibody. Radiotracer is bound to the antibody structure via -OH group of the tyrosine.

reaction mixture was gently agitated, the reaction alternatively might be or not stopped with 100  $\mu\text{l}$  of the solution sodium thiosulfate in water (4 mg/ml) (Chizzonite et al., 1991).

Iodination tubes, for both methods, were prepared in the same way. 100  $\mu\text{l}$  of iodogen dissolved in chloroform (10 - 500  $\mu\text{g}/\text{ml}$ ) was given in a glass tube and chloroform was evaporated under a slow stream of nitrogen. The prepared iodination tubes were used immediately. The procedure for the direct method consisted in adding 10  $\mu\text{l}$  of TU-20 (1 mg/ml) into the reaction tube with 50  $\mu\text{l}$  of phosphate buffer (PB, 0,05 M, pH 8,5) and an equal amount of  $\text{Na}^{125}\text{I}$  around 5,4 MBq. Reaction time was 15 minutes.

The indirect method was performed in two steps. Firstly, radioactivity in PB was added into the tube coated with iodogen. After 15 minutes an activated iodide was withdrawn, transferred into the vessel containing 10  $\mu\text{l}$  of the antibody and the mixture was agitated for 20 minutes (Švecová et al., 2008).

Radioiodination of the fragment scFv TU-20 was performed via chloramine-T without stopping reaction with thiosulfate as described previously for TU-20. In both cases, at the end of labeling, the reaction mixture was loaded on the top of a BSA-blocked polyacrylamide desalting column with an exclusion limit 6 kDa. Fractions were eluted with 0,1 % BSA in PBS and measured for radioactivity. (Hamilton, 2002), (Katsetos, 2003).

## 2.2 Immunoreactivity testing by enzyme linked immunosorbent assay (ELISA)

The immunoreactivity of the radiolabeled monoclonal antibody TU-20 was determined by an enzyme linked immunosorbent assay (ELISA) using the commercial set for detection of mouse anti -  $\beta$  III tubulin antibodies from VIDIA, CZ. One of the most useful of the immunoassays is the two antibody sandwich ELISA. This assay is used to determine the antigen concentration in unknown samples. This ELISA is fast and accurate, and if a purified antigen standard is available, the assay can determine the absolute amount of antigen in an unknown sample. The principle of ELISA testing is shown in the Fig. 3.

The sandwich ELISA requires two antibodies that bind to epitopes that do not overlap on the antigen. This can be accomplished with either two monoclonal antibodies that recognize discrete sites or one batch of affinity-purified polyclonal antibodies. To utilize this assay, one

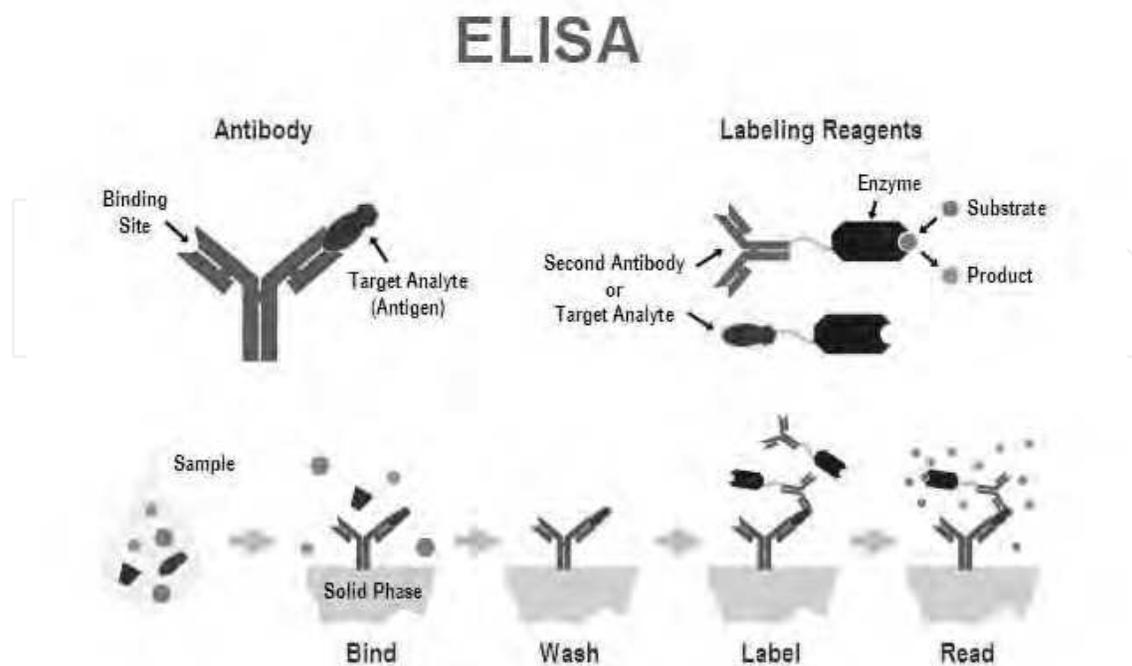


Fig. 3. ELISA principle. A specific antigen (an antibody plays the role of the "antigen" in the case of the antibody ELISA detection) is bound to the specific antibody coated on the solid carrier (microtitration plate). Subsequently, another specific antibody (labeled by an appropriate enzyme which catalyzes the coloured and easily detectable reaction) is added to the previously bound antigen.

antibody (the 'capture' antibody) is purified and bound to a solid phase typically attached to the bottom of a plate well.

Afterwards, an antigen is added, and, allowed to complex with the bound antibody. Unbound products are then removed with a wash, and a labeled second antibody (the 'detection' antibody) is allowed to bind to the antigen, and, therefore, the setting is described as the sandwich. The assay is then quantified by measuring the amount of labeled second antibody bound to the matrix, through the use of a colorimetric substrate.

Major advantages of this technique are that the antigen does not need to be purified prior to use, and that these assays are very specific. However, one disadvantage is that not all antibodies can be used. Monoclonal antibody combinations must be qualified as "matched pairs", meaning that they can recognize separate epitopes on the antigen so they do not hinder each other's binding.

ELISA procedures utilize substrates that produce soluble products. Ideally the enzyme substrates should be stable, safe and inexpensive. Popular enzymes are those that convert a colorless substrate to a colored product, e.g., p-nitrophenylphosphate (pNPP), which is converted to the yellow p-nitrophenol by alkaline phosphatase. Substrates used with peroxidase include 2,2'-azo-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), o-phenylenediamine (OPD) and 3,3',5,5'-tetramethylbenzidine base (TMB), which yield green,

orange and blue colors, respectively. In our case, TMB was used for colorimetric visualization. The settlement of the procedure see in the Fig. 4.

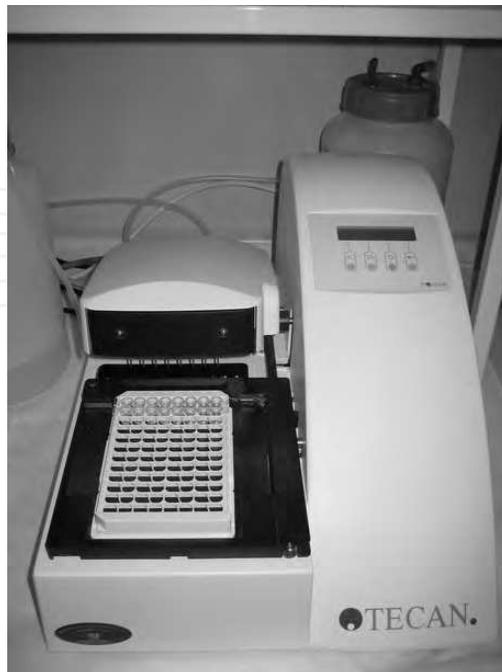


Fig. 4. Equipment for optical density measurement in the ELISA settings.

### 2.3 Immunoaffinity testing by radioimmunoassay (RIA)

In radioimmunoassay, a fixed concentration of radio-labeled antigen in trace amounts is incubated with a constant amount of antiserum such that the total antigen binding sites on the antibody are limited such that the only 30–50 % of the total radio-labeled antigen may be bound in the absence of the antigen. When unlabeled antigen, either as standard or test sample, is added to this system, there is competition between radio-labeled antigen and unlabeled antigen for the limited constant number of binding sites on the antibody.

The amount of radio-labeled antigen bound to antibody decreases as the concentration of unlabeled antigen increases. Following optimal incubation condition e.g. buffer, pH, time and temperature, radio-labeled antigen bound to antibody is separated from unbound radio-labeled antigen.

RIA analytic method was developed in two modifications of surface of the reactive vessel.

### 2.4 Immunoaffinity separation affinity coupling (AC)

Affinity coupling was developed by use the basic matrix activated Sepharose 4 Fast Flow by Pierce which was modified specific binding octapeptide (Vijayalakshmi, 1992). Activated media enable successful, convenient immobilization of ligands without the need for complex chemical syntheses or special equipment. The Sepharose matrix provides a wide range of high-capacity media with a variety of coupling chemistries for fast, easy, and safe immobilization through a chosen functional group. The principle is to immobilize the antibodies or other large proteins containing -NH<sub>2</sub> groups by coupling them to the matrix without the need for an intermediate spacer arm.

The correct choice of an activated medium is dictated by both the group available in the ligand molecule, and by the nature of the binding reaction with the substance to be purified. To ensure minimal interference with the normal binding reaction, immobilization should be attempted through the least critical region of the ligand (Haugland, 1995).

## 2.5 Stability testing by electrophoresis

Mostly used variation of the electrophoresis for the intention of the quality control of the radiolabeled substances is SDS-PAGE formation of the electrophoresis. It concerns of zone electrophoresis in gel in surface placement. The mixture of the substances is analyzed by division in accordance to the molecular weight.

### 2.5.1 Polymerization of the polyacrylamide gel

Polyacrylamide gel is prepared to the form by polymerization of the basic monomer acrylamide ( $\text{CH}_2=\text{CH}-\text{CO}-\text{NH}_2$ ; abbrev. AA) and  $N,N'$ -methylene-bis-akrylamid ( $\text{CH}_2=\text{CH}-\text{CO}-\text{NH}-\text{CH}_2-\text{NH}-\text{CO}=\text{CH}-\text{CH}_2$ ; abbrev. BIS) which is implemented to the polymere randomly and might covalently bind two linear chains of the polyacrylamide. Ammonium persulfate (abbrev. APS) is used as the initiative reactant and  $N,N'$ -tetramethyldiamine (abbrev. TEMED) as the catalyzer, see the Fig. 5.

The inhibitor of the reaction is oxygen, and, therefore, the gel must be protected against the oxygen atmosphere. The polymerization has the radical and exothermic process, and, therefore, the cooling is necessary during the whole polymerization. The ratio of AA:BIS is crucial for the gel mechanic and separation characteristics. The suggested ratio is ranging of about 40:1 (from 20:1 up to 100:1) (Jones, 2004).

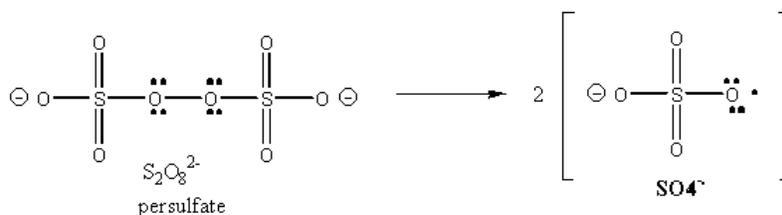


Fig. 5. The Free radical Polymerization of the Acrylamide Initiated on the Addition of the Ammonium Persulfate which Forms the Free Reactive Radicals in the Water.

### 2.5.2 PAGE electrophoresis

PAGE separation could be conducted in the gel with the same content of the acrylamide in two different following gels, so called Laemmli electrophoresis, when the first gel contents lower percentage of the acrylamide and it is intended to the concentration of the sample at the beginning of the separation (so called the concentration gel). The bigger sharpness of the zones in the gel is provided by means of the lower pH (of about two degree) against the surrounding setting. The itself separation takes place in the following part of the gel with the higher density (so called the separation gel). (Laemmli, 1970) The structure of the polymerization process see in the Fig. 6.

Other variation performs the creation of the gradient gel, where the concentration gradient of the polyacrylamide (from the part with lowe density to the part of higher density, in the

direction of the separation) is created. The bigger sharpness of the gel zones of the molecules of the similar size is ensured in this arrangement.

The choice of pH of the used buffer by polymerization process, and, also the division of the molecules by the classical PAGE, because, the suitable buffer ensures the sufficient differences in the specific charge of the assorted parts of the protein mixture. The acid proteins require slightly alkaline or neutral pH (the molecules moves to the anode) and alkaline proteins require a slightly acid pH (the molecules migrate to the cathode) (Bernard et al., 1979).

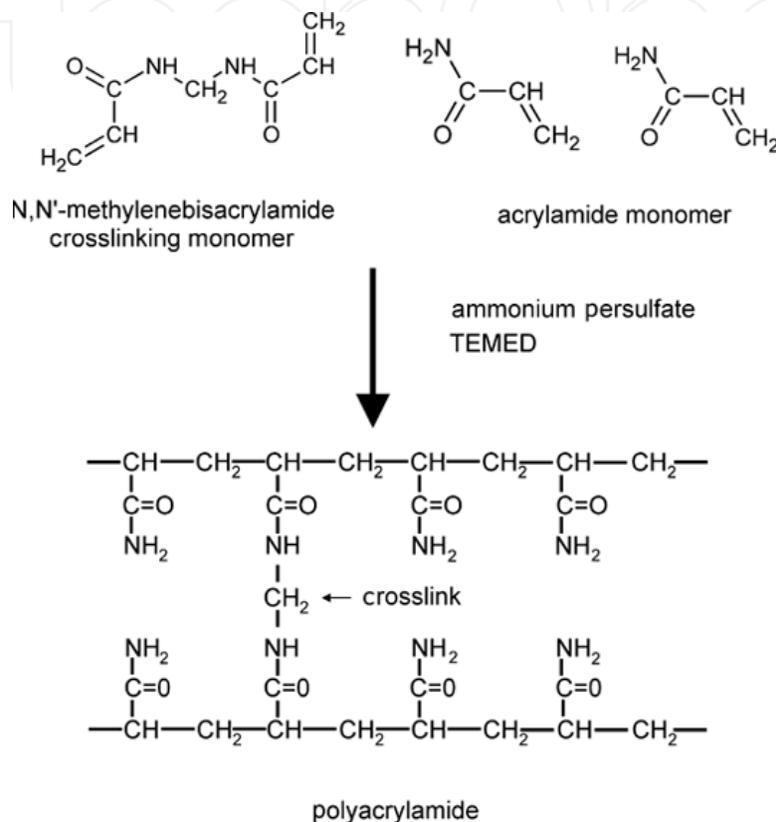


Fig. 6. Polymerization Process of the Structure Networking.

### 2.5.3 SDS-PAGE electrophoresis

The perfectly suitable modification of the PAGE electrophoresis is an arrangement in the –sodium dodecyl sulfate (abbrev. SDS, or NaDS), which makes an ability of the proteins to bind the SDS in amount of about 1,4 mg per 1 mg of the protein by means of the hydrophobic reaction. SDS carries a huge negative charge which enables to equalize the charge of the molecules, and, those, move in one direction in the electrophoretic gel in accordance of the molecular size. The complex SDS-protein unifies either the charge density, or, conformation on the surface of the complex, see the structure in the Fig. 7.

The mobility of the SDS-protein complex in the polyacrylamide gel is proportional to the logarithm of the molecular weight of an appropriate protein, which enables the gel calibration (Rédei, 2008). It is quiet convenient that the examined samples are adjusted before the whole process.

First, an appropriate buffer is added (e.g. TrisHCL) and SDS so that we have the same homogenous reaction setting.

Second, the glycerol is added, because it makes the settings in the gel more dense, so that the samples fill the sample holes properly and do not swirl. Glycerol also decreases the electroendosmosis and makes the movement and distribution of the proteins even better.

Third, the bromophenol blue is added as the protein movement indicator. Fourth, dithiothreitol (abbrev. DTT) could be added to cleave the proteins to make an analysis more suitable. The samples could be also denatured in the hot water by the temperature of about 65 °C.

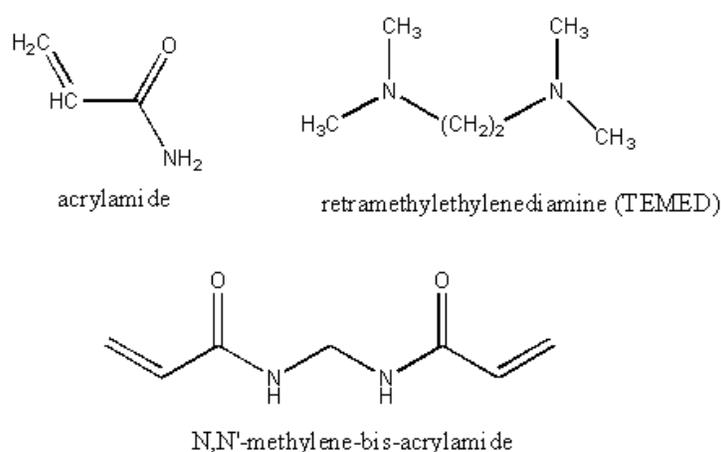


Fig. 7. The Structural Formulae of the Substances in the SDS-PAGE.

#### 2.5.4 Visualization and radiodetection in electrophoresis

The proteins can be visualized directly in gel after electrophoresis proceeding, or, subsequently Western Blot technique could be processed and detection is performed in the membrane where the proteins are transferred from gel. Adsorption of the pigment is used for visualization.

#### 2.5.5 Staining in electrophoresis

A Silver Staining shows another alternative for dyeing of the proteins in gel. The silver ion is insoluble and colourless, and, distinguishes the places with protein and without proteins in the polyacrylamide gel (formation of the silver complexes with alkaline or sulphuric proteins).

After this procedure, the silver ions are reduced by formaldehyde into the form of the metal silver which is perfectly visible and insoluble. The amount of proteins, which could be visualized by this procedure, ranges from the hundreds of picograms to the units nanograms.

Another staining, which is possible for this purpose of detection, is dyeing by means of Coomassie Blue which is less sensitive (of about 50 times), but it has another advantage that Coomassie Blue is bound to the protein in the stoichiometry ratio, and, therefore, it represents a quantitative densitometry detection (maximum absorbance ranges from 560 nm to 575 nm), see the Fig. 8. An autoradiography may be used as an alternative for detection in gel of the radiolabeled compounds. The differences between electrophoresis by non-reductive (see Fig. 9 and Tab. 1) and reductive conditions (see Fig. 10 and Tab. 2) are shown below.

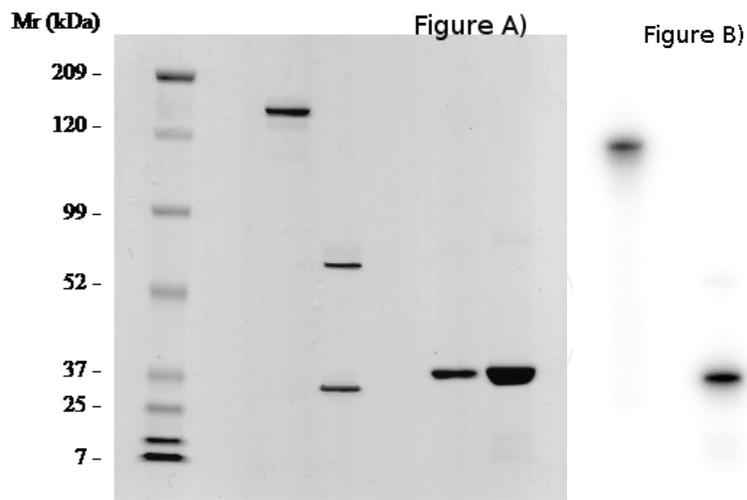


Fig. 8. Electrophoresis of the TU-20 and scFv TU-20 in the Gradient Gel by the Non-Reductive (-) and Reductive (+) Conditions Figure A) Gel Coloured by Coomassie Blue: 1. Molecular Marker; 2. TU-20 (-); 3. TU-20 (+); 4. scFv TU-20 (-); 5. scFv TU-20 (+). Figure B) Autoradiography: 1. [ $^{125}\text{I}$ ]TU-20 (-); 2. [ $^{125}\text{I}$ ]scFv TU-20 (-). .

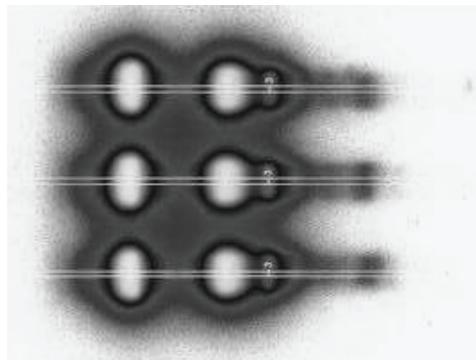


Fig. 9. Autoradiography of electrophoresis SDS-PAGE  $^{125}\text{I}$ -TU-20 (all lines) by non-reductive conditions

Peak	Integral Density in PSL	% Ratio of Peak
$^{125}\text{I}$ -TU-20	59237,2	46,7
BSA(I)	53228,4	42,0
BSA(II)	14319,4	11,3

Table 1. Autoradiographical interpretation of SDS-PAGE of  $^{125}\text{I}$  TU-20 by non-reductive conditions. An autoradiographical visualization of the SDS-PAGE gel (which contains the radiolabeled antibody by non-reductive conditions) after developing on the luminiscent plate by means of the AIDA software.

### 2.5.6 Immunoblotting

Western Blot transfers the proteins, closed into the gel matrix, into the nitrocellulose membrane for further purposes of investigation after finishing of electrophoresis. Western Blot (Immunoblotting), used for the protein detection, transfers the proteins from the gel into the membrane by means of electrophoresis.

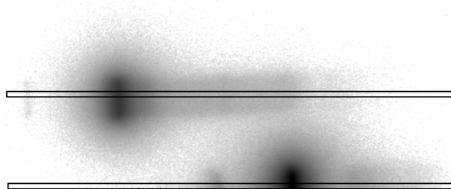


Fig. 10. Autoradiography of electrophoresis SDS-PAGE of  $^{125}\text{I}$ -TU-20 and  $^{125}\text{I}$ -scFv TU-20 by reductive conditions. An autoradiographical visualization of the SDS-PAGE gel (which contains the radiolabeled antibody and fragment by reductive conditions) after developing on the luminiscent plate by means of the AIDA software.

Peak	Integral Density in PSL	% Ratio of Peak
$^{125}\text{I}$ -TU-20	63445,2	98,1
BSA(I)	1228,8	1,9
$^{125}\text{I}$ -scFv TU-20	77988,6	97,6
BSA(I)	1909,7	2,4

Table 2. Autoradiographical interpretation of SDS-PAGE of  $^{125}\text{I}$ -scFv TU-20 (upper line) and  $^{125}\text{I}$  TU-20 (bottom line) by reductive conditions.

The particular proteins are subsequently indentified by the appropriate radiolabeled antibodies (labeled by enzymatic reaction, or, by the radiolabeling reaction with  $^{125}\text{I}$ ). The proteins bound into the membrane could be submitted to the non-specific staining, or, as an alternative, to the autoradiography. After drying, the membrane is stored with much better results than dried gel.

When the electrophoresis with all its instruments and alternatives is used as a quality control method of the radiolabeled antibodies, the following parametres were proved and chosen for this setting as the most suitable. Stability of the radiolabeled TU-20 and its scFv TU-20 was investigated on 4 - 12 % Bis-Tris gel electrophoresis.

Protein bands were visualized by staining the gels with Silver Stain Plus.  $^{125}\text{I}$ -labeled scFv fragment was processed by autoradiography exposing plate BAS-SR 2025, and finally developed by BAS-1800II. Autoradiographs were evaluated by AIDA 2.0 software, see the Fig. 11.

## 2.6 Immunohistochemistry testing

Preserved binding properties of the radiolabeled MAb or scFv for neuronal tissue were confirmed by the method of double labeling. It is based on the immunohistochemistry and autoradiography of the brain tissue slices. The 50  $\mu\text{m}$  thick brain slices from the wild type mouse (C57B/6/J) were incubated with the radiolabeled TU-20. The second incubation was performed with anti-mouse IgG polyclonal antibody conjugated with horseradish peroxidase (Sigma-Aldrich, USA). Afterwards, the immunohistochemistry was finalized by staining with 3,3' - diaminobenzidine (DAB) that revealed the neuronal structure, see the Fig. 12 and 13.

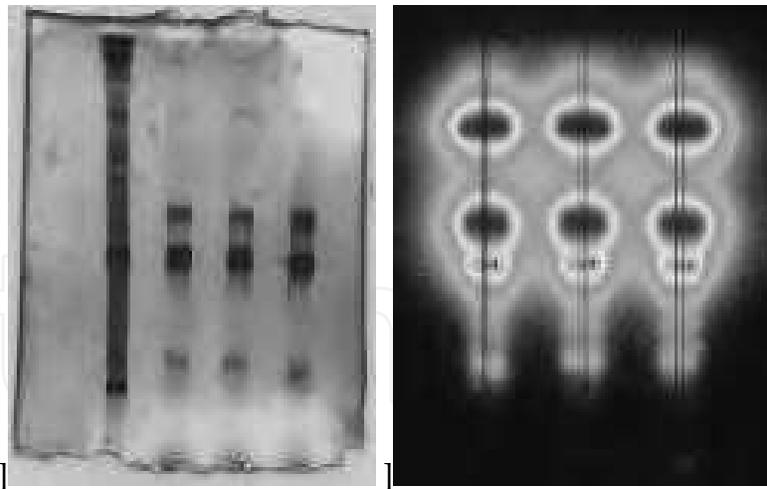


Fig. 11. Gel electrophoresis analysis of [ $^{125}\text{I}$ ]TU-20 – autoradiography (a) and silver staining (b).

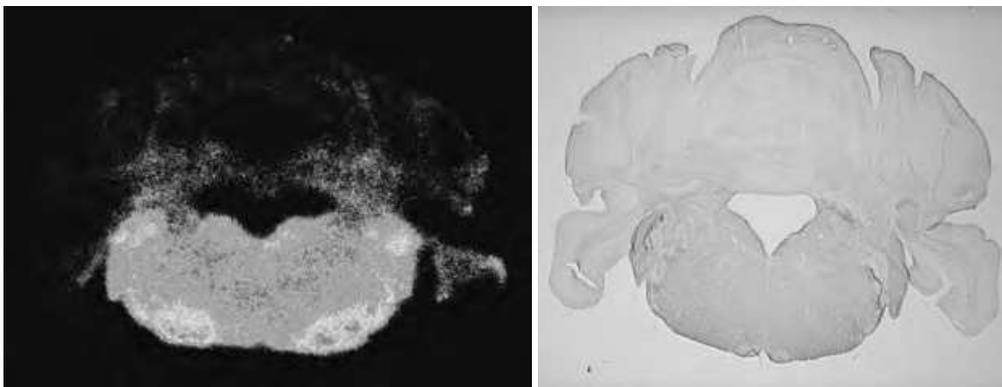


Fig. 12. [ $^{125}\text{I}$ ]TU-20 autoradiographical - Figure A) ,and, immunohistochemical visualization of the bound radiolabeled antibody in the mice brain slice - Figure B) image of the coronal mice brain slice.

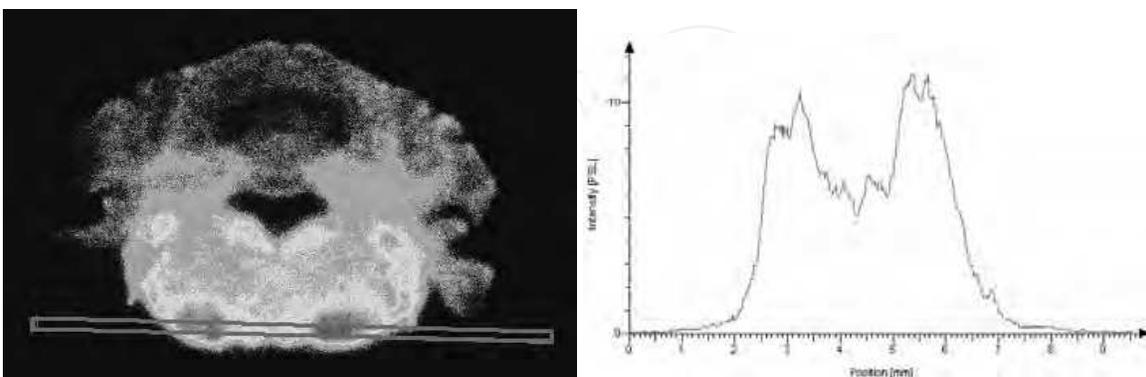


Fig. 13. Autoradiography visualization - Figure A), and, visualization of the 1D-interpretation of the bound radiolabeled antibody in the mice brain slice - Figure B) of the labeled mice brain slices by means of the software AIDA.

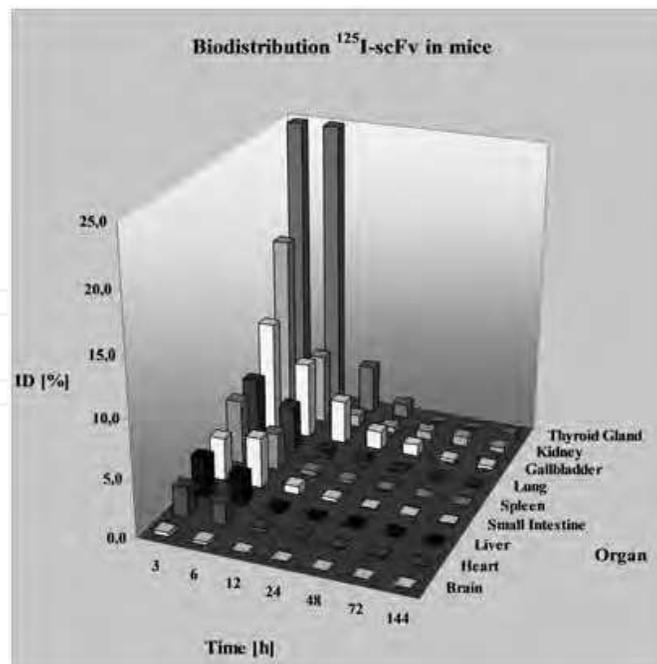


Fig. 14. [ $^{125}\text{I}$ ]scFv biodistribution in normal mice.

## 2.7 In vivo preparative biodistribution testing in normal mice

The in vivo biodistribution was carried out with the male normal mice - wild type C57B/6/J. Biodistribution studies were performed following an i.v. injection. The main focus is intended for scFv fragment due to its better mobility in organism.  $^{125}\text{I}$ -labeled scFv fragment, for comparison with the biodistribution of  $\text{Na}^{125}\text{I}$ , was applied in amount of 50 kBq/50  $\mu\text{l}$ .  $^{123}\text{I}$ -labeled scFv fragment was injected in amount of 200 kBq/50  $\mu\text{l}$ .

Mice were sacrificed at designated time points in groups by 3 animals. The kinetic time intervals were: 3, 6, 12, 24, 48, 72, 144 hours for  $^{125}\text{I}$ -labeled scFv TU-20 fragment and 0,5, 1, 2, 3, 6, 12 hours for  $^{123}\text{I}$ -labeled scFv fragment.

Blood and major organs (included thyroid gland, kidneys, lung, heart, brain, spleen, muscle, fat, skin, gallbladder, testicles, stomach, liver, small intestine, and colon) were removed, weighed, and counted in a gamma scintillation counter to determine the % ID/g (percentage of injected dose per gram) for each radiolabeled substance.

The biodistribution figures are shown below, see the Fig. 14 and 15.

Blood clearance data for  $^{125}\text{I}$ -labeled scFv fragment were obtained by analyzing blood samples by using a bi-exponential model for two-phase clearance to determine short phase half-life  $t_{1/2\alpha}$  and long phase half-life  $t_{1/2\beta}$  values.

## 2.8 In vivo SPECT imaging biodistribution testing

[ $^{123}\text{I}$ ]scFv TU-20 and [ $^{123}\text{I}$ ]TU-20 behavior in mice (wild type C57B/6/J) was observed by use of the SPECT camera. Kinetic intervals were 0,5, 1, 2, 3 h by [ $^{123}\text{I}$ ]scFv TU-20 - see the Fig. 16 and 1, 2, 3, 6 h by [ $^{123}\text{I}$ ]TU-20 - see the Fig. 17.

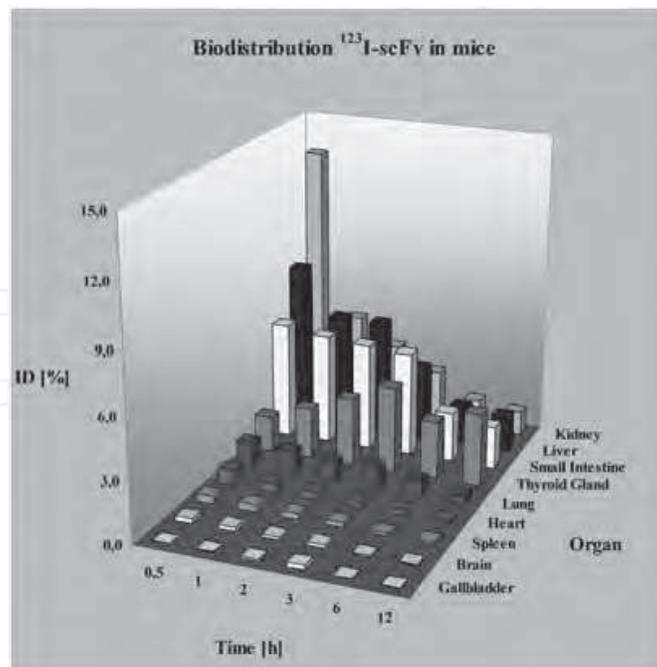


Fig. 15.  $^{123}\text{I}$ scFv biodistribution in normal mice.



Fig. 16.  $^{123}\text{I}$ scFv TU-20 SPECT camera images –biodistribution study in kinetic intervals 0,5, 1, 2 and 3 h.

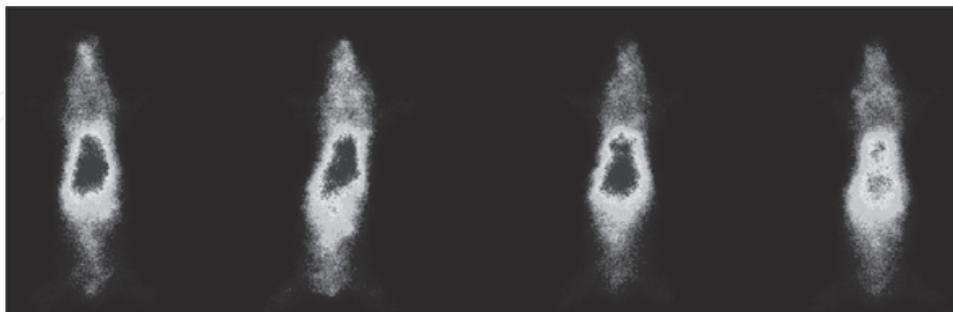


Fig. 17.  $^{123}\text{I}$ scFv TU-20 SPECT camera images –biodistribution study in kinetic intervals 1, 2, 3, 6 h.

### 2.9 In vivo biodistribution testing in genetically modified mice

Transgene population G93A1 Gur was used for comparative study to show different behavior of the substances in normal mouse and in modified organism with amyotrophic lateral

sclerosis (ALS). Biodistribution kinetic intervals were 3 h ( $^{125}\text{I}$ -scFv) and 6 h ( $^{125}\text{I}$ -TU-20). (Heiman-Patterson T.D., 2005) (Naini A., 2007)

### 3. Conclusion

TU-20 and its scFv were labeled with  $^{125}\text{I}$  and  $^{123}\text{I}$  by chloramine-T (with average yield 0,72 and 0,50, resp.). Radiochemical purity and stability was revealed by gel filtration (decrease to 80 % and 50 % in two months, resp.) Fragmentation of the labeled antibody and its fragment was estimated by bis-tris gel electrophoresis followed by silver staining and autoradiography (over 95 % of radioactivity bound in the substances).

Affinity coupling and RIA adaptation for the specific conditions showed 10-30 % preserved immunoreactivity of the labeled compounds. Otherwise, these methods carry out quite high discrepancy and it will be necessary to provide further optimising search.

In vitro studies performed on mice brain slices confirmed several important assumptions. The antibody is preferentially bound in the layer of Purkinje cells in the cerebellum. SPECT camera in vivo experiment deals with these results: activity bound in scFv is primarily distributed to the thyroid gland and digestive tract, then passes quickly through kidneys.

Distribution images of the labeled TU-20 provides ambiguous because the substance is accumulated in the chest and ventral part and image resolution do not afford more detailed biodistribution identification. However, it is known from previous biodistribution preparative study that activity is distributed in lung, heart, liver, stomach and colon in first 6 h.

In vivo experiments were focused on investigation of the blood clearance and organ distribution of the radiolabeled TU-20 and scFv fragment in mice. Let's show especially the results from scFv biodistribution study in preference. It was verified that the major part of activity, according to the amount of the labeled scFv fragment, was eliminated from blood during 2-3 hours. Minor part of activity, according to the amount of the labeled scFv fragment (0,5 - 1,0 %), was kept in the blood for some days. The value  $t_{1/2\alpha}$  for  $^{125}\text{I}$ -labeled scFv fragment was calculated as 2,3 h and the  $t_{1/2\beta}$  was estimated as 62,4 h. The half-life for overall elimination of  $\text{Na}^{125}\text{I}$  from blood was 4,5 h.

In comparison, we found that the  $^{125}\text{I}$ -labeled scFv fragment uptake in thyroid gland appeared much lower than for  $\text{Na}^{125}\text{I}$ , as expected. The  $t_{1/2\alpha}$  value for  $^{123}\text{I}$ -labeled scFv fragment was calculated as 1,4 h, but the long phase elimination half-life  $t_{1/2\beta}$  was not estimated due to short half-life of the isotope  $^{123}\text{I}$ . The radiolabeled scFv fragment passed in general through the digestive tract (stomach and intestine) and finally was eliminated through kidneys in preference.

TU-20 and ScFv TU-20 showed suitable properties for further investigation in animals which are genetically modified mutants with the ALS (Amyotrophic Lateral Sclerosis). Comparing biodistribution experiments in modified organism confirmed expected behavior. The most significant biodistribution differences occurred in the area of the limbs and caudal part of spinal cord and spine.

Finally, as I can summarize, TU-20 and its scFv fragment were successfully labeled with radioiodine  $^{123}\text{I}$  and  $^{125}\text{I}$ , and, subsequently, the biochemical and analytical characteristics were investigated. Biological properties of the radiolabeled TU-20 and its scFv were evaluated in vivo by biodistribution studies.

The expected behavior of biomolecules during their elimination was observed. Furthermore, the elimination parameters were calculated.  $^{125}\text{I}$ -labeling of the TU-20 and its scFv is very suitable for investigation of the radiolabeled antibody fragment behavior and properties due to the long  $^{125}\text{I}$  half-life. On the other hand,  $^{123}\text{I}$ -labeling of the scFv fragment TU-20 is intended for practical imaging at SPECT camera.

In summary, TU-20 shows better immunospecific behavior in organism together with slower kinetics, on the other hand, scFv TU-20 reveals worse immunospecific characteristics in combination with much faster kinetics.

#### 4. Acknowledgement

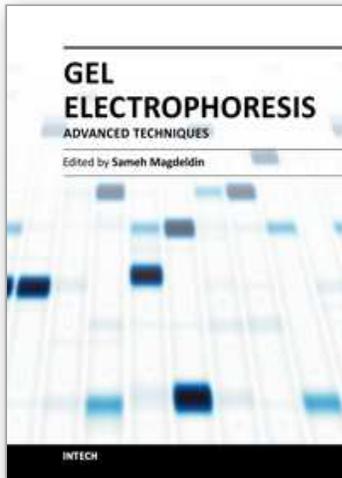
This work was supported by the projects No. E!3177 - DIAGIM (1P040E167) and E!2510 - NEUROTUB (0E91) of EUREKA, and by the project No. IBS1048301 of Grant Agency CAS.

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URL: <http://dx.doi.org/10.1385/0-89603-213-2:33>

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## **Gel Electrophoresis - Advanced Techniques**

Edited by Dr. Sameh Magdeldin

ISBN 978-953-51-0457-5

Hard cover, 500 pages

**Publisher** InTech

**Published online** 04, April, 2012

**Published in print edition** April, 2012

As a basic concept, gel electrophoresis is a biotechnology technique in which macromolecules such as DNA, RNA or protein are fractionated according to their physical properties such as molecular weight or charge. These molecules are forced through a porous gel matrix under electric field enabling uncounted applications and uses. Delivered between your hands, a second book of this Gel electrophoresis series (Gel Electrophoresis- Advanced Techniques) covers a part, but not all, applications of this versatile technique in both medical and life science fields. We try to keep the contents of the book crisp and comprehensive, and hope that it will receive overwhelming interest and deliver benefits and valuable information to the readers.

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