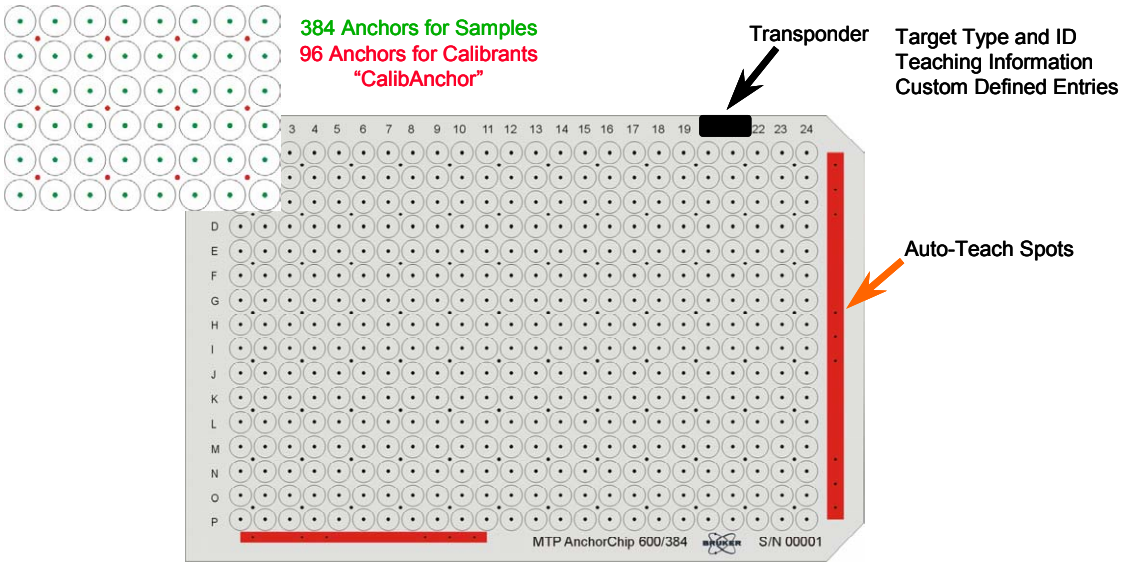
AnchorChip™ Technology

Revision 2.0

Preparation for Ultra-Sensitive Automated MALDI-TOF MS



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List of Abbreviations

2,5-DHB	2,5-Dihydroxybenzoic acid
3-HPA	3-Hydroxypicolinic acid
HCCA	α-Cyano-4-hydroxycinnamic acid
SA	Sinapinic acid

1 General Description

AnchorChip™ is a patented* new micro preparation technique designed for ultra-sensitive automated mass spectrometric (MALDI) analysis.

AnchorChip™ targets are equipped with hydrophilic patches ("anchors") in hydrophobic surroundings.

Typically 0.5-3 µl liquid is deposited onto the anchors. Sample droplets shrink during solvent evaporation and center themselves onto the anchor positions. Thus the anchors can be regarded as wells without walls. Anchor diameters between 200 µm and 800 µm can be placed at the disposal suitable to cover nearly all conceivable experiments.

AnchorChip™ targets can be washed and are reusable many times.

The hydrophobic AnchorChip™ coating is not only „phobic" against water but also against almost all relevant organic solvents. Alcohol, acetonitrile and even acetone have been used successfully.

Numerous adapted AnchorChip™ preparation protocols for the most common MALDI matrices are described in this manual. Simple on-target washing and recrystallization procedures help to remove water soluble contaminants.

In contrast to the conventional dried-droplet preparation method on (hydrophilic) metal targets, the AnchorChip™ technology benefits in:

- Enhancing the detection sensitivity by a factor of 10 to 100 because of the concentration of the analyte to the center, the "Anchor". This feature yields finally in an increased data output of the related sample.
- Reducing a great deal the search for "sweet spots" in order to get good mass spectra. Applying the AnchorChip™ technique the samples are located at predefined positions, with crystals covering the entire sample area. This facilitates and speeds up the automatic data acquisition even with water-soluble matrices.

AnchorChip™ technique takes advantage of a true micro preparation, however without the additional need for nano-pipetting devices, which also might be applied, if available. Standard µl-pipetting robots (e.g. Bruker MAP II) or even manual preparation is sufficient.

The basic principle of AnchorChip™ vs. dried-droplet preparation and a size comparison of typical prepared samples is shown in figure 1.

* Brukers AnchorChip™ Technology is protected by German patent DE 197 54 978.0 and US patent US6287872. Patents applied for elsewere.
Licensed in Germany for end users by Sequenom under German Patent application DE 197 82 096.4 and the branched off utility model DE 297 24 252.0.

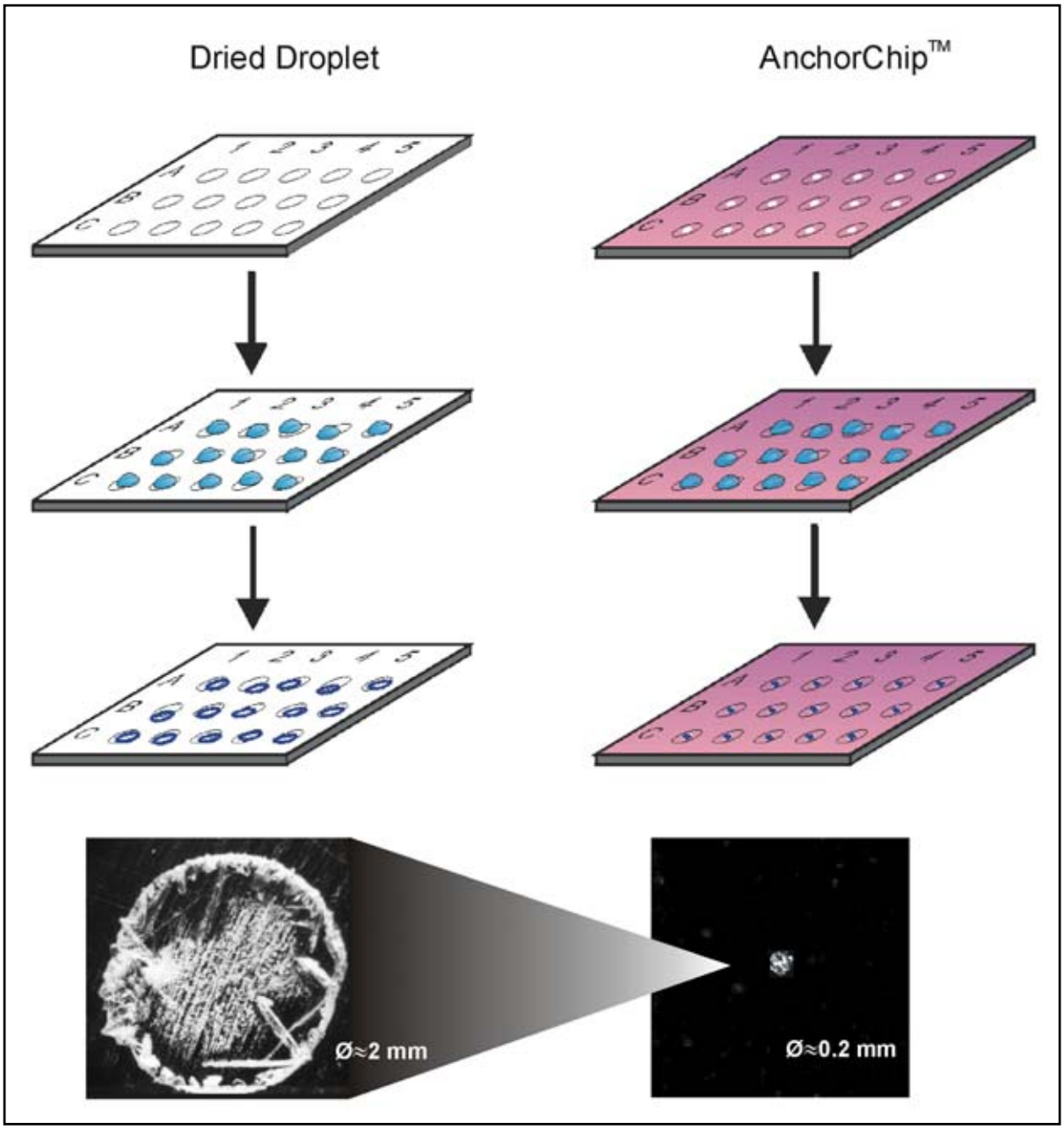


Fig. 1: The basic principle of AnchorChip™ vs. dried-droplet preparation for 2,5-DHB matrix

Usually dried-droplet preparations employed for water-soluble MALDI matrices (e.g. 2,5-DHB, 3-HPA) on conventional metal sample supports, typically result in samples with an irregular crystalline rim (1-2 mm diameter) and a micro crystalline area in the center. Here good mass spectra can only be obtained irradiating the rim, and a time consuming search for "sweet spots" is often necessary.

2 What Anchor Size is Best for My Application?

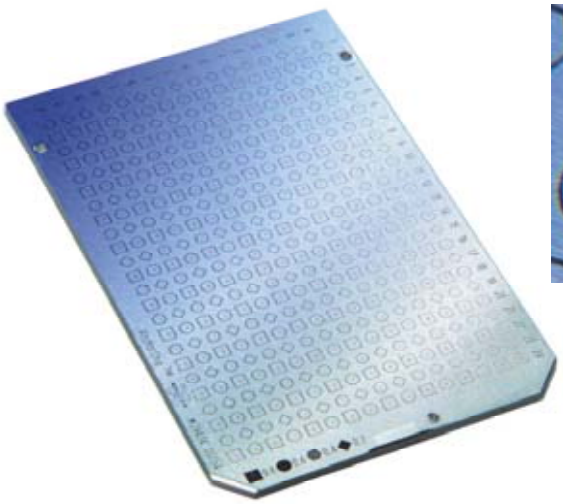
400 µm	... for water soluble matrices 2,5-DHB, 3-HPA.
600 µm	... for water **in**soluble matrix HCCA, SA.
Mixed size	... to figure out the best anchor size.

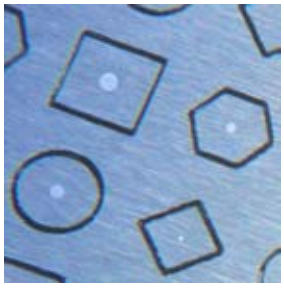
200 µm and 800 µm are **not** recommended for standard applications.
200 µm anchors are not able to attract µl droplets reliably.

There exists a direct correlation between the anchor size and sensitivity because of the anchor size dependent matrix load.
Preparations on larger anchors (600/800 µm) provide only a moderate sensitivity increase compared to standard dried-droplet preparations. However the bigger the size the bigger is the tolerance towards the impurities because of the higher matrix load.
Water-soluble matrices form a crystalline rim on large anchors. This rim is precisely located at the hydrophobic/hydrophilic borderline and is therefore most suitable for automated runs.

Larger anchors are generally better suited for „difficult samples“ (e.g. DNA 100mer with 3-HPA) where the „in-situ“ purification of spatially separated crystals may be preferred.

The maximum sensitivity increase is observed on smaller anchors (200/400 µm) because of the reduced matrix amount and the resulting higher molar analyte/matrix ratio.
On the other hand, low amounts of matrix material require more skill and expenditure of time as well to prevent impurification while preparing the target.
Water-soluble matrices do not form a crystalline ring on smaller anchors but a crystalline agglomerate covering the anchor area instead. Sweet spot searching is usually not necessary in this case.





AnchorChip™ var/384
Part No. 73023 (209515 with transponder)
4 x 96 anchors of different size (200-800 µm).
This target can be used to find out which anchor size is best for a specific application.

3 Cleaning the AnchorChip™

AnchorChip™ targets are reusable many times.

The coating on AnchorChip™ targets is characterized as follows:

- Mechanically robust. Wiping (not chafing!) and sonication are allowed. **Non-contact sample deposition** methods are mandatory.
- Resistant against organic solvents (esp. alcohol, acetonitrile, acetone...).

CAUTION: NEVER USE BASES OR ACIDS!
THEY CAN DESTROY THE COATING.

We recommend the following washing procedure prior to each preparation:

1. Wipe the target with a suitable solvent on a tissue (e.g. acetone for HCCA).
2. 5-10 min. sonication with MeOH/H_2O 1:1 (v/v).
3. Rinse with MeOH.
4. Rinse with demineralized H_2O.
5. If you want to store washed targets, we recommend to repeat steps 3 and 4 prior to usage.

- **Do not put the target frame into the washing buffer**, because it dries very slowly. Poor vacuum would be the result!
- **Use always the same anchors for your calibrants**. It is always difficult to remove high amounts of analyte completely (memory effect).

Regeneration

After a number of applications the hydrophobic characteristics of the coating may decrease because traces of detergents remain to the surface. To overcome this problem in most cases, the function of the coating can be restored by wiping and/or incubation in a non-polar solvent, e.g. n-hexane.

Performance check of the hydrophobic coating

1. The surface should be strongly water repellent. Water droplets (100 µl should roll easily over the surface without any visible tailing. Little water droplets should be visible on the anchor areas after flushing the targets with water.
2. Even acetone should not spread out, but should form island-like droplets on the surface instead. When tilting the target slightly, these islands should move with low tailing only.

4 Sample Preparation on AnchorChip™ Targets

AnchorChip™ preparation protocols significantly differ from conventional dried-droplet preparations.

The most obvious reason for this is the fact that the AnchorChip™ preparations are much smaller than conventional dried-droplet preparations. Therefore the **matrix concentration should be reduced by 1:5-1:50**, compared to those on conventional metal surfaces. Too much crystallized matrix material may result in a "tower".

The second major difference is the droplet shrinking on the AnchorChip™. Upon solvent evaporation the analyte concentration, but also the concentration of any contaminant present in the initial droplet increase dramatically.

To get started please follow strictly the rules and protocols given below.
In case of problems please refer to the troubleshooting paragraph 10.

4.1 General Rules

1. **Many plastics are not compatible with AnchorChip™** because they release polymers. Problems with unsuitable plastics typically result in strange looking crystallization and/or polymer signals in the spectra. In extreme cases samples do not crystallize at all and remain liquid even after hours.
   Avoid all kind of siliconized tubes. On the other hand even some "pure" polypropylene tubes / tips / MTPs release polymers. We strongly recommend to use brands which have been proven to be compatible with AnchorChip™.
   Please refer to the list in paragraph 9.

2. **Solvent and matrix purity is of critical importance!**
   Note: Not only the analyte but also any impurities present in the droplets are concentrated during solvent evaporation. If solvent impurities cause problems, volume reduction or dilution in pure solvents may help.
   Please refer to the recommendations given in paragraph 9.
   Avoid to use detergents. They temporarily eliminate the hydrophobic effect of the coating and prevent the droplets from shrinking. To restore exhausted AnchorChip™ targets, please refer to paragraph 3.

3. Also the **biochemical processing of the analyte** before MALDI preparation is crucial. Be aware of detergents, contaminants or polymer releasing plastics. For in-gel digests a protocol developed by Shevchenko has proven to be compatible with AnchorChip™.
   Shevchenko et al., Anal. Chem. 68 (1996) 850-858.

On-target washing and recrystallization help in some cases to get rid of salts and contaminants. Adapted protocols (HCCA) are given in paragraph 4.2.3.
AnchorChip™ technology is compatible with C18 **ZipTip™** sample purification (http://millipore.com), provided the organic solvent concentration in the elution buffer does not exceed 50 %, otherwise you may get polymer peaks from ZipTip™ bed material.

4.2 Adapted Protocols for 2,5-DHB / SDHB (super DHB)

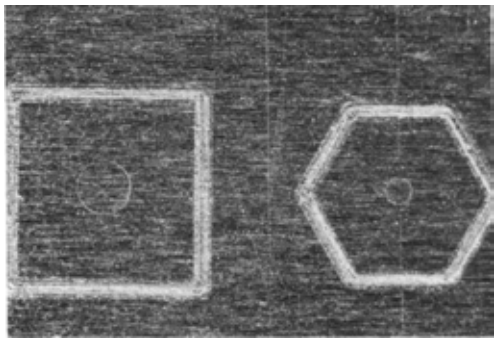
2,5-DHB (2,5-dihydroxybenzoic acid) is a commonly used MALDI matrix for peptides and proteins.
It can easily be used on AnchorChip™, even dissolved in water with no addition of organic solvent.

2,5-DHB is more robust against impurities (e.g. 3 mM Tris-HCl or 10 mM ammonium bicarbonate) than HCCA but gives slightly worse external calibration results due to the complex 3D surface topography of 2,5-DHB crystals.

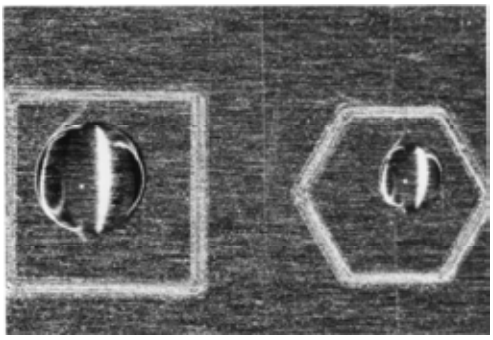
Protocol **0.5 µl 2,5-DHB (5 g/l in H_2O or 1:2 acetonitrile : 0.1 % TFA) + 0.5-1 µl analyte solution on 400 µm AnchorChip™.**

- The 400 µm anchor should be well-covered with matrix crystals (fig. 2). Microscopic inspection is recommended.
- You can either apply the matrix solution first and let it crystallize before adding the analyte solution or you can apply a matrix / analyte premix.
- Molar matrix amount depends on anchor size.
  Crystalline rim preparation can be achieved on 600/800 µm anchors (use 0.5-1 µl 1-10 g/l 2,5-DHB).
- If the sample does not crystallize properly you can increase the molar amount of matrix to compensate the influence of impurities.
- For **proteins** we recommend to use "**super DHB**" (**SDHB**) instead of 2,5-DHB. This matrix contains an isomeric additive which improves the high mass performance. Order information can be found in paragraph 9.
  Protein applications require higher organic solvent concentrations in the initially prepared droplets because hydrophobic domains of the proteins tend to stick to the hydrophobic AnchorChip™ coating.

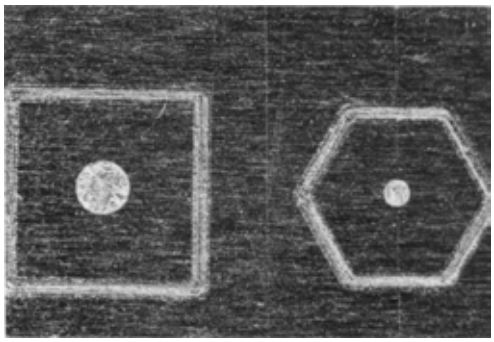
The pictures shown in fig. 2 on the next page demonstrate the typical procedure for 2,5-DHB / SDHB AnchorChip™ preparations.



400 / 800 µm anchors
Note: The anchors are the small circles inside the inscriptions!



Shrinking droplets
after deposition of 1 (0.5) µl 2,5-DHB
on 800 (400) µm anchor.



2,5-DHB crystals on the anchors.

2,5-DHB preparation on 800 µm anchor.
Note: crystalline rim.

2,5-DHB preparation on 400 µm anchor.
Note: Anchor completely covered with crystals.

Fig. 2: 2,5-DHB preparation on AnchorChip™

4.3 Adapted Protocols for 3-HPA

3-HPA (3-hydroxypicolinic acid) is a widely used MALDI matrix for oligonucleotide samples (DNA/RNA). Like 2,5-DHB, it is water-soluble. However, 3-HPA cannot be applied as easily as 2,5-DHB since oligonucleotide samples tend to form (multiple) alkali adducts. To avoid this, the **targets have to be cleaned very carefully**. We recommend to flush the targets with plenty of demineralized water in addition to the standard washing procedure (paragraph 3).

Protocol

Matrix solution: 3-HPA (10 g/l) + DAC* (1 g/l) in H_2O
Apply 0.5-1 µl matrix solution onto 400 µm anchor.
Let the matrix crystallize.
Add 0.5-1 µl analyte solution onto the matrix crystals.

** Diammonium hydrogen citrate (e.g. Fluka Part No. 09833)*

- The addition of **DAC** helps to suppress alkali adduct formation.
- 3-HPA AnchorChip™ preparations without DAC look pretty much the same as 2,5-DHB preparations (fig. 2). With the addition of DAC matrix crystals look more milky than 2,5-DHB crystals. As for 2,5-DHB, 400 µm anchors should be well-covered with matrix. Crystalline rim preparation on 800 µm anchors is recommended for difficult samples.
- We recommend to **aliquot the matrix** solution and to store the aliquots in the **freezer**. Use fresh aliquots daily.
- Optionally you can incubate the matrix solution and / or the DNA solution with **NH_4^+-cation exchange beads** for at least 10 minutes, preferably overnight *(e.g. Biorad PolyPrep AG50W-X8, Part No. 731-6213, prior to use the H^+-form has to be converted into the NH_4^+-form).*
- For DNA MALDI purposes, use **demineralized water** with specific resistance better than 18 MΩcm only!
- **Dust seems to be the main origin for external Na / K contamination.** Please ensure that, upon drying, the dust intake in the matrix / analyte droplets sitting on the anchors is minimized. Decreasing the droplet volumes (short drying time!) or working in a cleanbench can help to reduce alkali adduct ion formation. Note: Cigarette smoke is one of the worst K pollutants!
- The Bruker magnetic bead purification kit (**genopure™**) is a powerful tool for DNA sample purification. It works both on manual and robotic bases. For detailed information please refer to Bruker Application Note #MT-55 where MALDI genotyping using AnchorChip™ and genopure™ is described.

4.4 Adapted Protocols for HCCA

HCCA (α-cyano-4-hydroxycinnamic acid) is a widely used MALDI matrix for peptides, especially in Proteomics applications.

Since HCCA is water-insoluble, special protocol adaptations are required. The following example helps to illustrate the general problem of water-insoluble matrices in combination with the AnchorChip™ technology.

Imagine that 1 µl HCCA dissolved in 50 % acetonitile is applied onto an anchor. The organic solvent evaporates first, and below a certain acetonitrile concentration the HCCA precipitates. Unfortunately, this usually happens before the droplet has shrunk onto the anchor. In order to circumvent this problem, the initial concentration of the organic solvent is drastically increased. On the other hand, very poor MALDI results are obtained if too much organic solvent is used (crystallization rate is too high!).

Despite of this tricky multi-parameter balance (org. solvent concentration, applied volume, molar matrix amount, anchor size), the following protocols are robust and give nice results even for in gel digests.

Note: The simple "thin layer affinity" protocol described in paragraph 4.4.3 can only be used if the analyte solution does not contain any organic solvent.

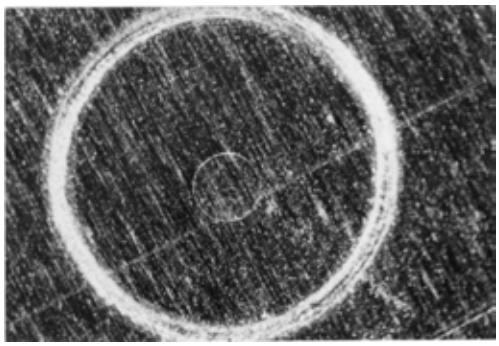
4.4.1 "Standard" HCCA AnchorChip™ Preparation with Optional On-Target Washing and Recrystallization

Protocol **Matrix solution: HCCA (0.3 g/l in EtOH : acetone 2:1)**
prepare from stock solution HCCA (1 g/l in acetone)
Apply 0.2-1 µl analyte solution *together* with
1-2 µl matrix solution onto 600 µm anchor.
Let the sample crystallize at room temperature.

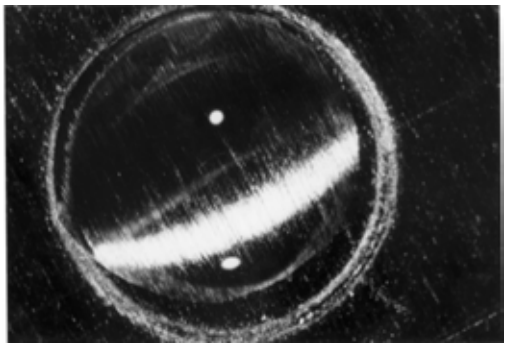
- Use suitable plastics only! (Strictly obey rules given in par. 4.1)
- Prepare matrix solutions daily.
- The analyte solution may contain 0-70 % organic solvent.
- If you apply 0.2 µl analyte solution only, the sample will shrink to the anchor as shown on the pictures in fig. 3. A **dense layer of tiny well-separated crystals** covering the anchor should be visible under microscopic inspection (fig. 3e).

➢ The more water is contained in the initially prepared droplet, the earlier the matrix crystallization starts. If you get crystals outside the anchor area, we recommend sample **Recrystallization** (see below).

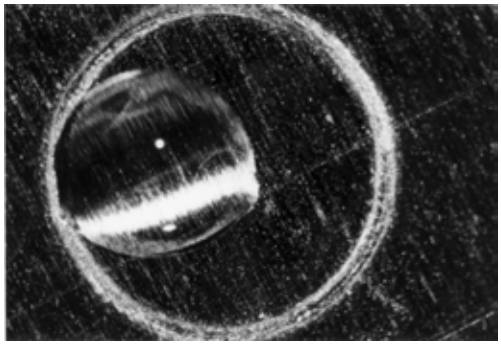
➢ If you work with real life samples (e.g. in gel digests +buffers) you might get a "ill" looking crystallization. **On-target washing and recrystallization** is recommended in this case (see below).



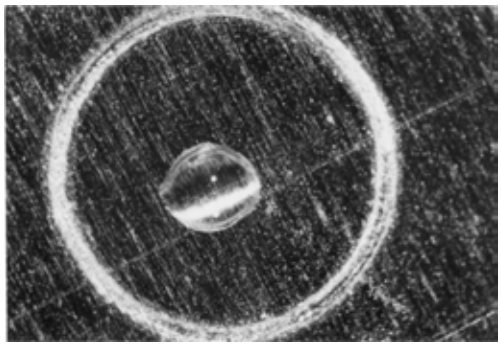
a) 600 µm anchor
Note: The anchor is the small circle inside the inscription!



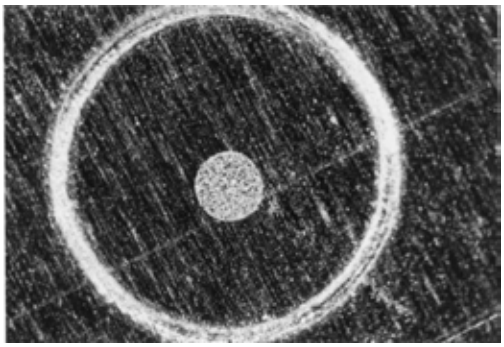
b) Deposition of 2 µl HCCA matrix solution and 0.2 µl analyte solution (30 % acetonitrile).



c) Shrinking droplet



d) Shrinking droplet



e) HCCA preparation on the 600 µm anchor.

Fig. 3a-e: "Standard" HCCA AnchorChip™ preparation

ON-TARGET WASHING (optional)

On-target washing is a simple procedure to remove water soluble contaminants. HCCA crystals with the incorporated analyte will not dissolve.

Protocol **Apply 1-5 µl 0.1-1 % TFA onto the sample.**
Remove washing buffer after a few seconds with the pipette tip.
Do not touch the crystals with the tip.
Let the residual liquid evaporate.

The on-target washing procedure is shown in fig. 4a-c.

RECRYSTALLIZATION (optional)

Recrystallization redissolves matrix crystals and brings the preparation onto the anchor, independent of the initial droplet composition.

Protocol **Apply 0.5-1 µl of EtOH : acetone : 0.1 % TFA (6:3:1).**
Let the sample dry.

The recrystallization procedure is shown on fig. 4d-f.

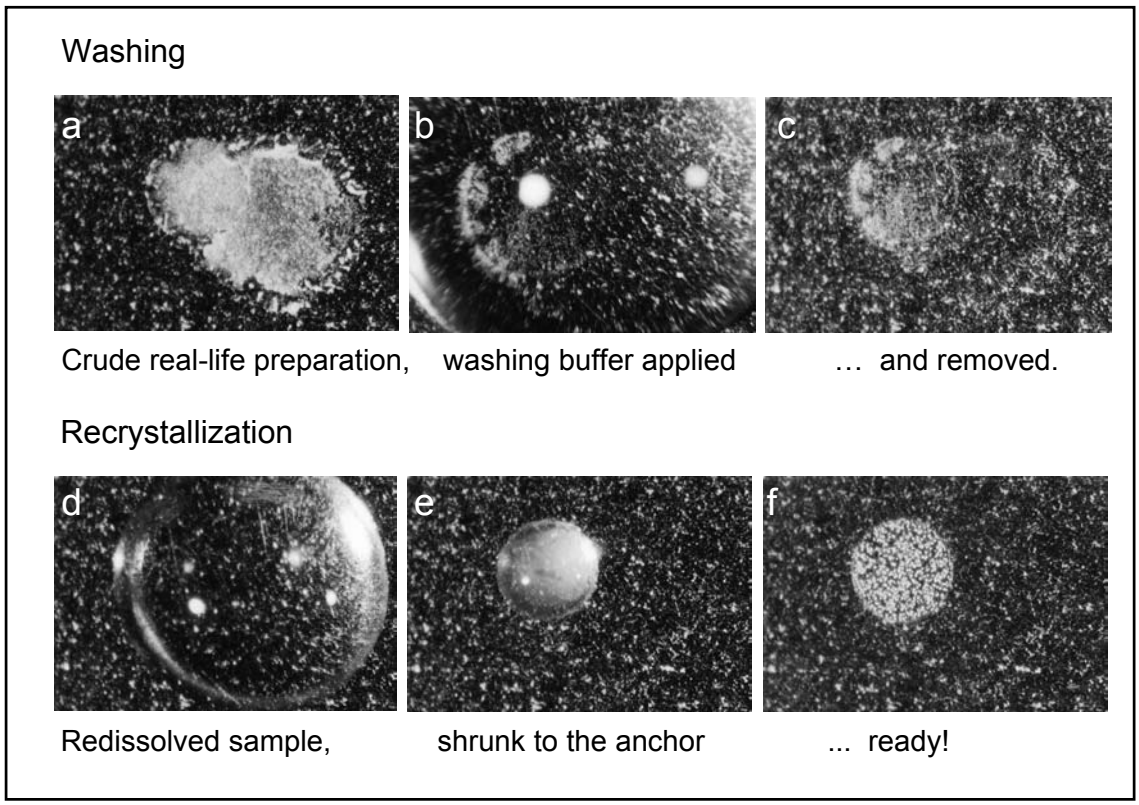


Fig. 4a-f: Washing and recrystallization of a HCCA preparation containing 0.5 µl 50 mM ammonium bicarbonate on 600 µm anchor (see text).

4.4.2 Alternative "Standard" HCCA AnchorChip™ Preparation Protocol

- Matrix solution: HCCA (0.2 g/l, solvent: acetonitrile).
- Analyte solution: solvent acetonitrile : 0.1 % TFA (1:2)
- Apply 0.5 µl matrix/analyte premix (5:1) onto 400 µm or 600 µm anchor and let the solvent evaporate.
  Do not apply the matrix solution first and let it crystallize before adding the analyte solution. This will decrease spectra quality significantly.

  A dense layer of tiny well-separated crystals covering the anchor should be visible under microscopic inspection (fig. 3e). If a significant number of crystals are located outside the anchor area, decrease the water content in the premix or recrystallize the sample. If the density of crystals is too low, increase the HCCA concentration.

4.4.3 Thin Layer Affinity HCCA AnchorChip™ Preparation

The essence of this preparation

An **aqueous** droplet containing analyte and contaminants (salt, buffer…) is deposited on an anchor covered with HCCA thin layer. The analyte molecules attach to the HCCA thin layer whereas the water soluble contaminants stay in the liquid phase and are removed together with the residual droplet after a few minutes.
(Gobom et al., Anal. Chem. 73, 2001,434-438)

Using this method no further purification (e.g. ZipTip™) is required even for crude real life samples. The thin layer affinity preparation is the method of choice for in-gel digests if the tryptic fragments are eluted from the gel plugs with no organic solvents.

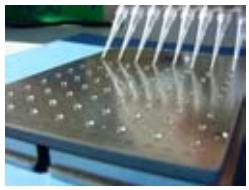
Many more laser shots per spot can be applied (important for MS/MS) after recrystallization of the thin layer.

Preparation Protocol

Matrix solution	HCCA sat. in acetone : 0.1 % TFA , 97:3 sonicate, centrifuge, take supernatant only!
Recrystallization Mix	HCCA (0.1 g/l in EtOH : acetone : 0.1 % TFA, 6:3:1)
Analyte solution	**MUST NOT** contain any organic solvent. (otherwise HCCA thin layer would be redissolved !) Addition of 1 mM *n*-octyl glucopyranoside sometimes help to increase signal intensity of high mass peptides.

1. Apply about 1-2 µl matrix solution onto 400/600 µm anchor and remove it with the pipette tip immediately.
   The anchor takes what it needs and after 1 second the HCCA thin layer is formed (see figure).

2. Deposit 1-4 µl aqueous analyte solution onto the thin layer anchor preparation and let it incubate for about 3 min. (see figure).
   It is essential that the sample must not dry on the thin layer.



3. Add 2-4 µl 0.1 % TFA to the excess liquid and remove the whole droplet as completely as possible with the tip.
   There is no chance to remove the liquid completely because the anchor always retains 10-100 nl. The addition of 0.1 % TFA before removing the analyte droplet helps to dilute contaminants.

4. Add 1 µl recrystallization mixture.
   The recrystallization mixture redissoves the thin layer with the attached analyte molecules, and adds some more HCCA in order to form larger crystals which allow more laser shots per spot.

4.5 Adapted Protocols for SA

SA (sinapinic acid) is a widely used MALDI matrix for proteins.

Protocol	**Matrix solution: SA (1 g/l in acetonitrile / 0.1 % TFA 90 :10)** **Apply 0.5-1 µl analyte solution *together* with** **0.5-1 µl matrix solution onto 600 µm anchor.** **Let the sample dry.** **Wash with ca. 10 µl 0.1-1% TFA (optional).** **Recrystallize with 0.5 µl matrix .**

- Note: Protein applications require higher organic solvent concentrations in the initially prepared droplets because hydrophobic domains of the proteins tend to stick to the hydrophobic AnchorChip™ coating.
- This protocol was developed for smaller proteins (<20 kDa). Modifications might be necessary for larger proteins.

5 Instrument Teaching of the Anchor Positions

Although the anchor raster itself is consistent and accurate, a slight rotation and an offset of the sample carrier which is accommodated in the tray may occur. Teaching corrects all these deviations from the ideal positions.

The teaching procedure gains a positioning accuracy of about 60 µm for all anchors.

General recommendation:
An unloaded anchor may be hardly visible on the monitor, depending on the target illumination. In such a case it is recommended to prepare a matrix spot on the calibration positions to enhance the anchors visibility.

5.1 FlexControl 1.1+ (MTP AnchorChips)

Please refer to the FlexControl online help.

AutoTeaching
Some 384 MTP AnchorChips (see list in paragraph 8) allow for fully automated teaching of the anchor raster in AutoFlex and UltraFlex. Please refer to the FlexControl online help.

5.2 Unix TOF Software 5.0+ (MTP AnchorChips)

In AutoXecute load the geometry file "384_AnchorChip". In order to do the teaching select the "Teach Calib Positions" and center the appropriate three anchor positions under the laser spot using the arrow keys.

In order to change the teaching positions edit the geometry file (e.g. /home/tof/automation/5.0/geometry_files/384_AnchorChip.geo") sand adapt the lines "First Calibrant", "Second Calibrant" and "Third Calibrant", respectively. The default positions are A1, A24 and P1.

After having done the 3 point teaching it is may be a good idea to save the target geometry under a different filename, preferably the unique target ID printed near the bottom right corner of each target.

5.3 AnchorChip™ for Scout26 Ion Source (Unix)

The 3-point teaching of a AnchorChip™ 25 or 36 (see list in paragraph 8) for the Scout26 ion source is done in an analogue way as described in paragraph 5.2. Please ignore the center position if asked for (just press OK).

6 CalibAnchors

Some 384 AnchorChip™ targtes are equipped with additional 96 extra anchors for improved external calibration (see list in paragraph 8).

This “CalibAnchor” configuration benefits in:

- performing external calibration in the direct neighborhood of the sample spot saving thus precious space,
- allowing most accurate measurements because possible slight curvatures of the target surface can be neglected.

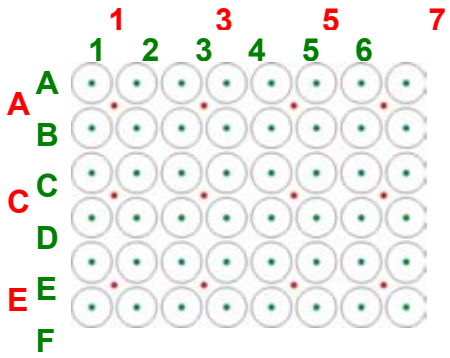
Sample and calibration anchors are grouped in a manner, that always four sample anchors surround one calibration anchor. Therefore only 96 positions out of possible 384 calibration anchors are required. The distance of the calibration anchors is exactly 9 mm, so that the Bruker’s robot MAP II/8 can be employed for preparation, when available. The arrangement of sample and calibration anchors is shown in the figure.

To differentiate between both kinds of anchors for manipulation the user can toggle between two chips:

Chip #1 is always reserved for calibration purposes whereas chip #0 is used for measuring samples.

Note: On chip #1 only 96 anchors are used for calibration, but 384 are displayed.

To prepare an automated run taking advantage of the CalibAnchors, the MS-Excel template *384measure_96calibrate* can be used that comes with the installation CD.



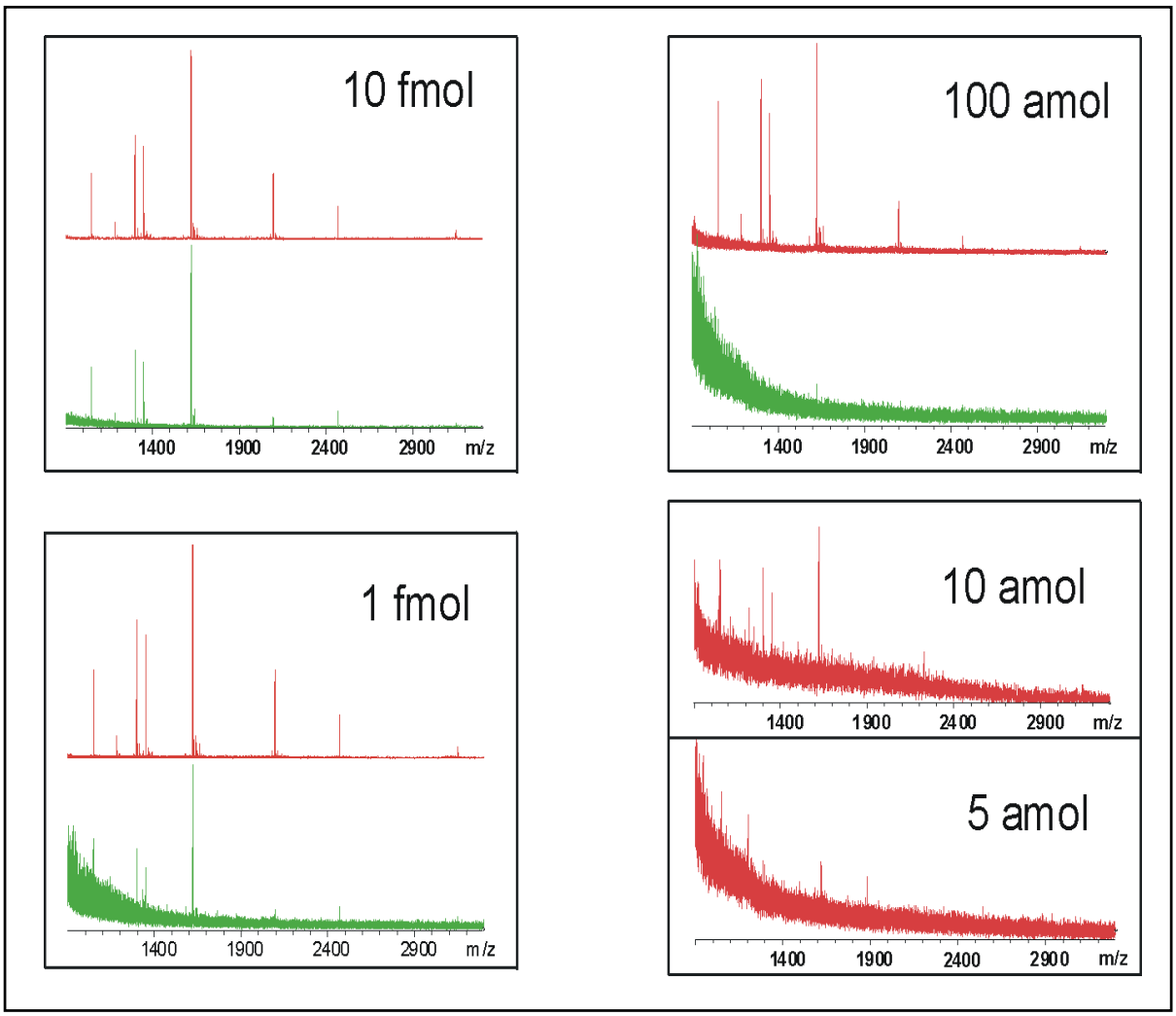
CalibAnchor layout

Chip #0: 384 sample anchors
Chip #1: 96 calibration anchors

7 Examples

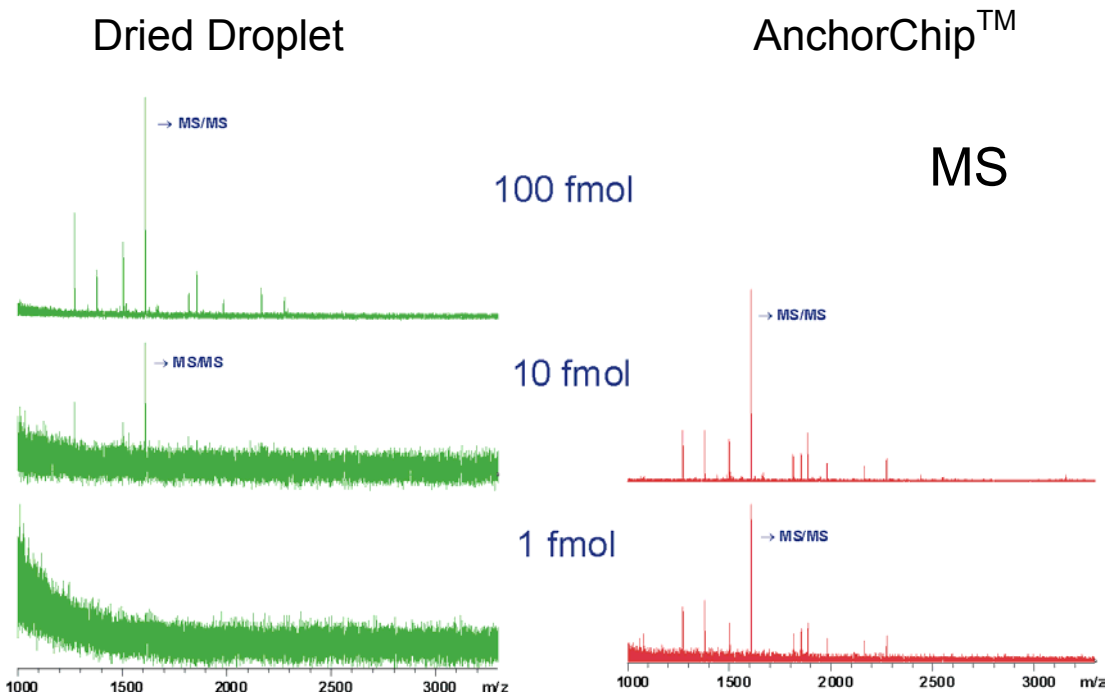
7.1 Peptide Mix

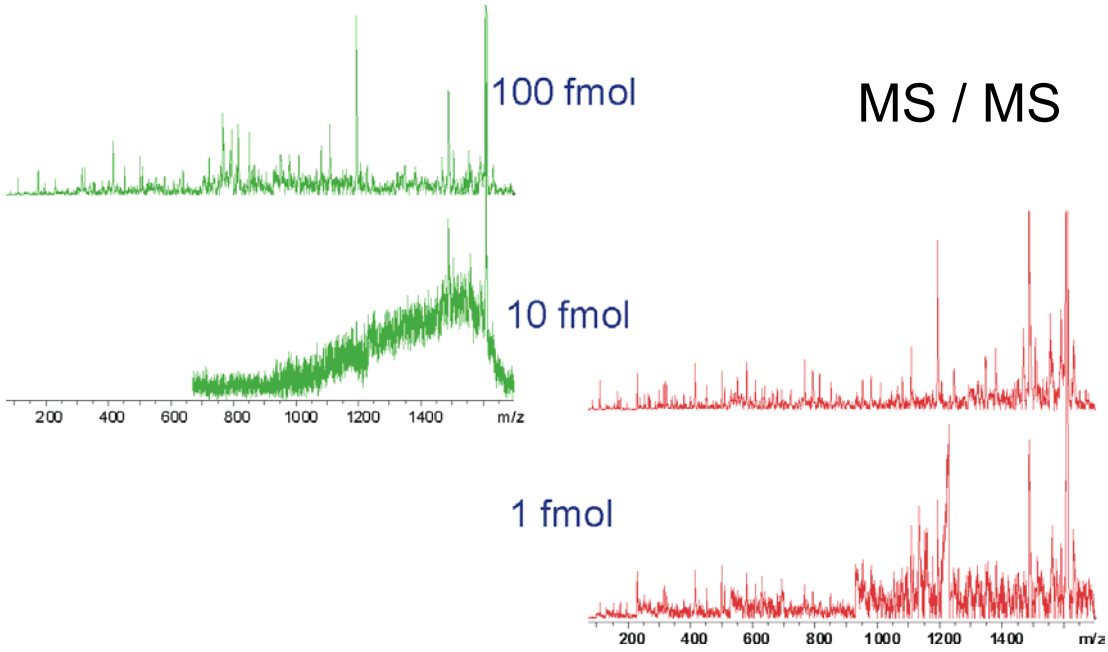
Analyte: Bruker peptide calibration standard (Part No.: 206195)
AnchorChip™ vs. dried-droplet preparation
Matrix: HCCA
Anchor: 400 µm



7.2 Peptide Map and MS/MS Sensitivity

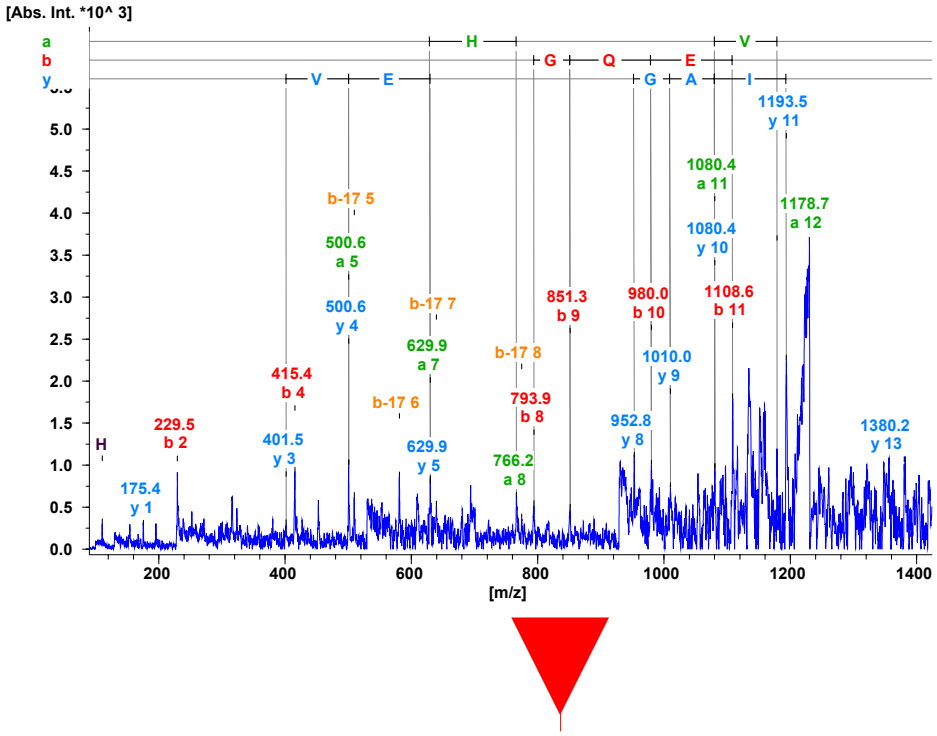
Analyte: tryptic myoglobin digest
Matrix: HCCA
Anchor: 400 µm
Tryptic fragment selected for MS/MS: m/z 1606.8 Da

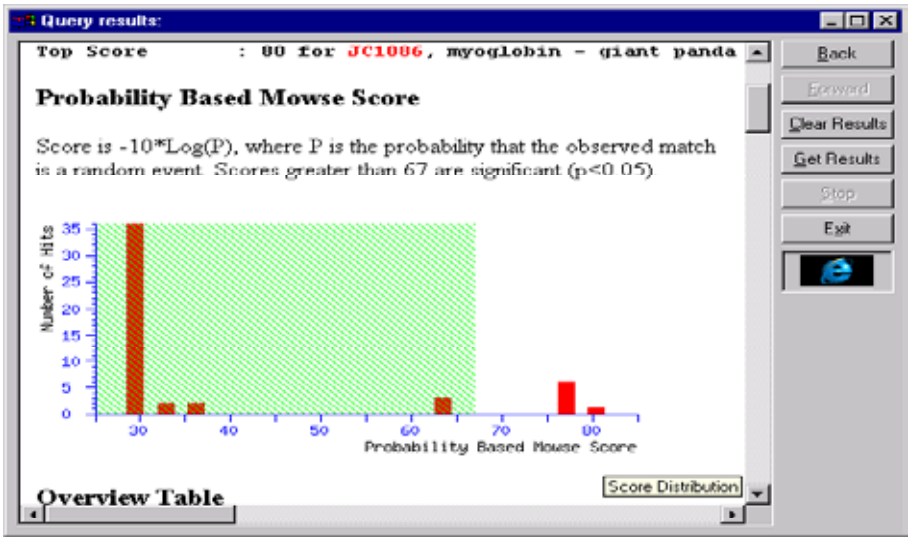




7.3 1 fmol MS/MS Protein Identification

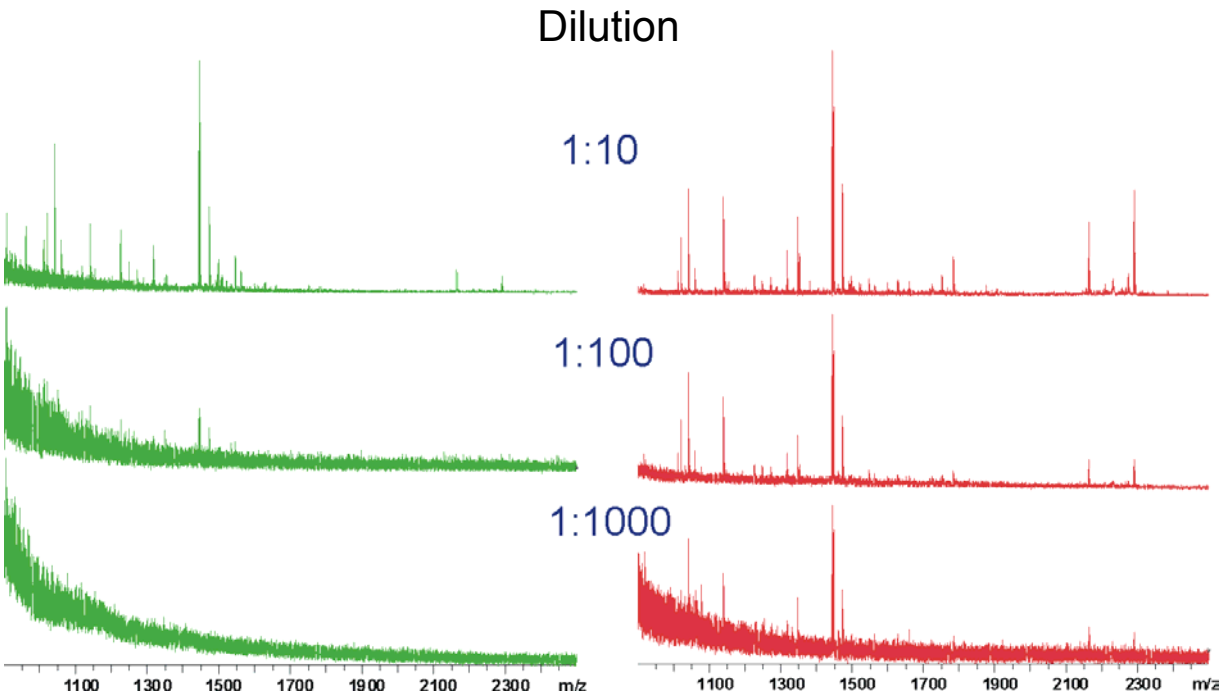
Analyte : tryptic myoglobin digest (1 fmol)
Matrix: HCCA
Anchor: 400 µm
Spectrum: compare Example 6.3
MS BioTools™ & Mascot Search





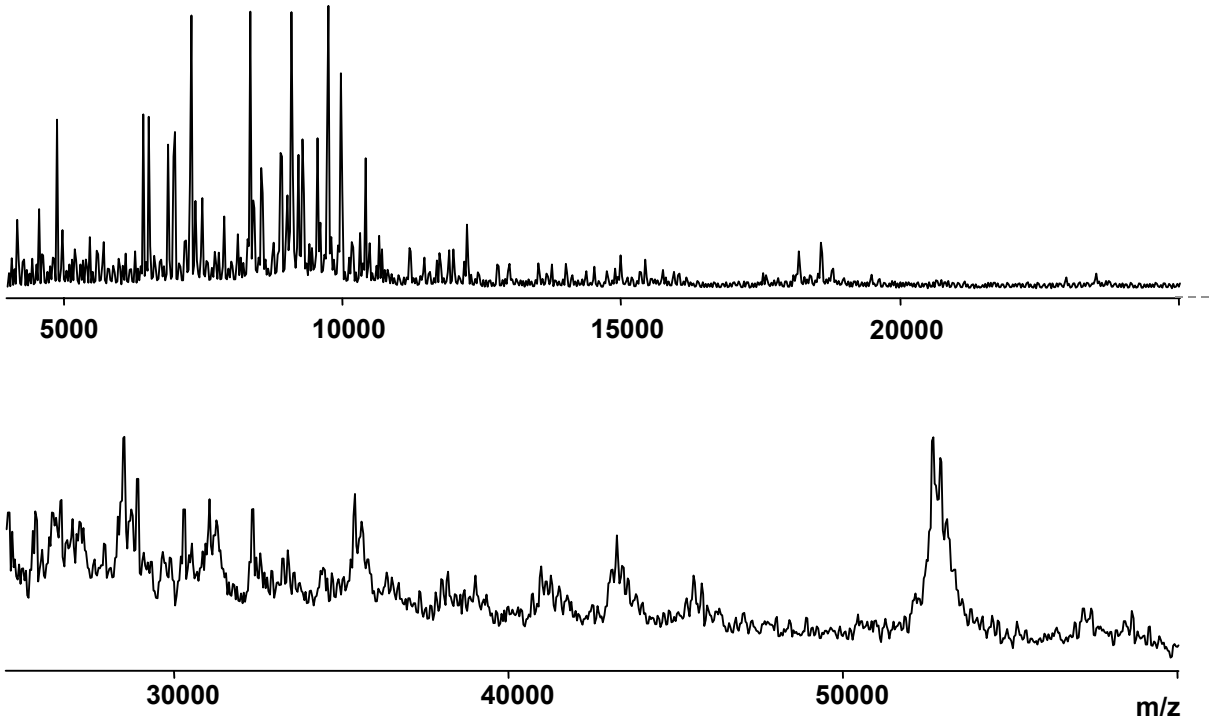
7.4 In-Gel Digest

Matrix: HCCA
Anchor: 400 µm



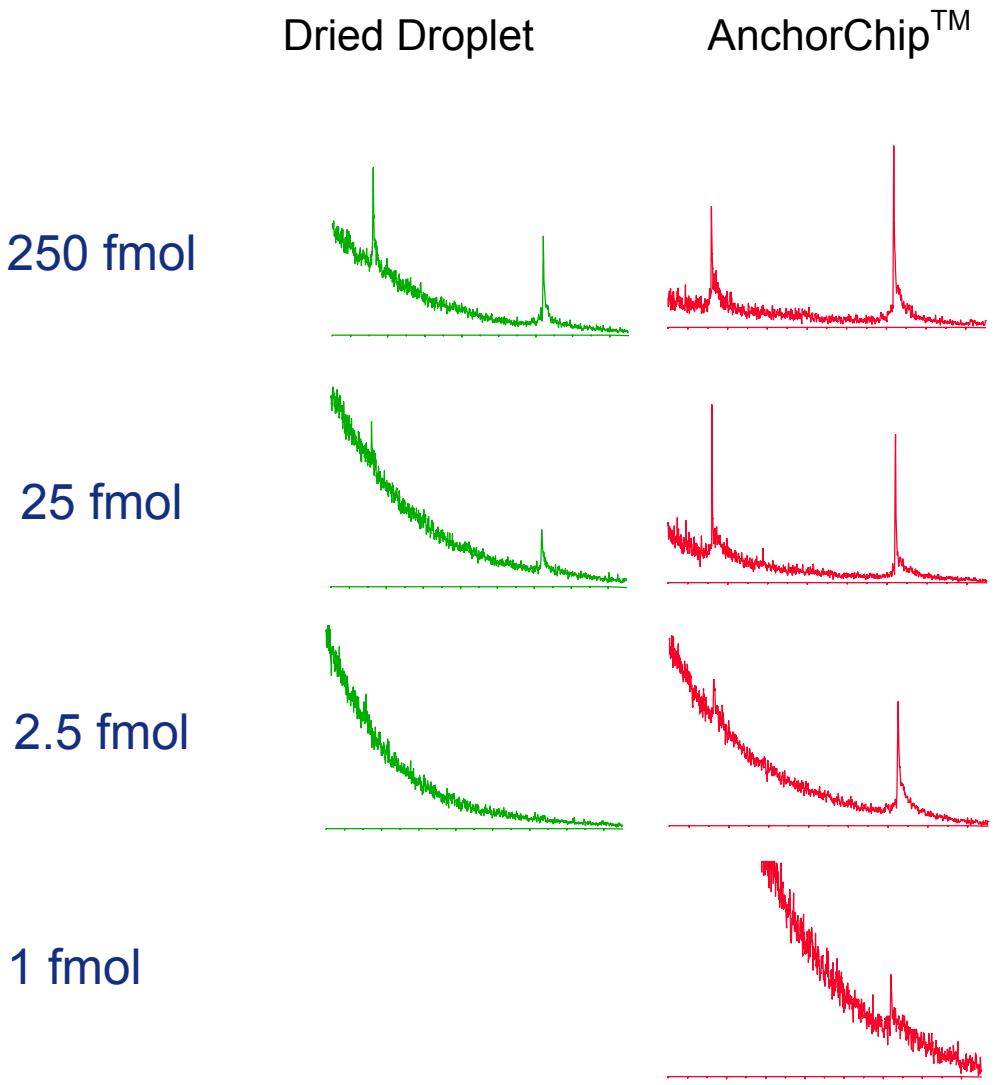
7.5 Unprocessed E.Coli Cell Extract

Matrix: Sinapinic acid (SA)
Anchor: 200 µm
Note: AnchorChip™ provides 20 times higher sensitivity than dried-droplet (data not shown)



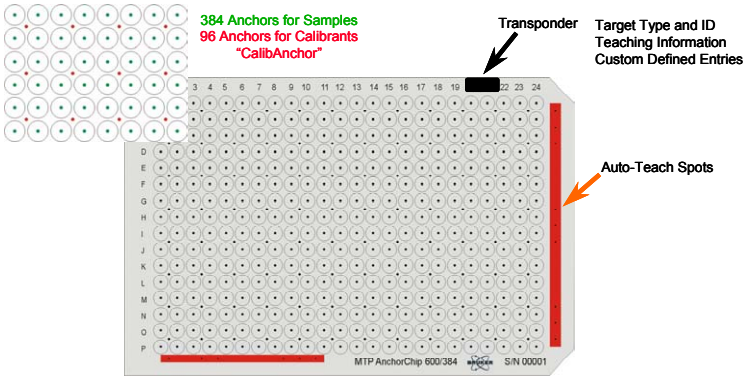
7.6 DNA 30mer

Analyte: synthetic DNA oligo 30mer
Matrix: 3-HPA
Anchor: 200 µm



8 AnchorChip™ Product Overview

MTP AnchorChips



New!

CalibAnchor
96 Extra Anchors for superior external calibration (see par. 6)

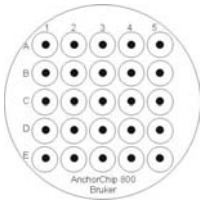
AutoTeaching
Fully automated teaching of the anchor raster in AutoFlex and UltraFlex (see par. 5.1)

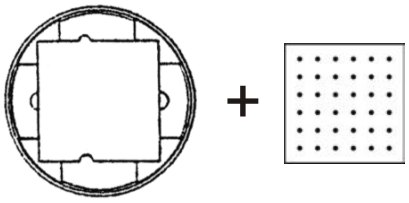
Transponder

Part No.	Type	Anchor Diam.[µm]	No. of Anchors	Calib. Anchor	Auto-teaching	Transponder	Frame Part No.
With Transponder							
209511	200/384 T	200	384	Yes	Yes	Yes	74115
209512	400/384 T	400					
209513	600/384 T	600					
209514	800/384 T	800					
209515	var/384 T	200-800		No			
209516	200/1536 T	200	1536				
209517	400/1536 T	400					
209518	600/1536 T	600					
Without Transponder (Will phase out!)							
73019	200/384	200	384	No	No	No	74115 or 204741
73020	400/384	400					
73021	600/384	600					
73022	800/384	800					
73023	var/384	200-800					
73215	200/1536	200	1536				
73216	400/1536	400					
73217	600/1536	600					

Scout26/100 AnchorChips

Part No.	Type	Anchor Diam.[µm]	No. of Anchors	Frame Part No.
One Piece (no frame required !)				
74110	200/25	200	5x5	No
74111	400/25	400	5x5	No
74112	600/25	600	5x5	No
74113	800/25	800	5x5	No
Inserts (to be mounted on adapter frame #14526)				
72431	200/36	200	6x6	14526
72432	400/36	400	6x6	14526
72433	600/36	600	6x6	14526
72434	800/36	800	6x6	14526





9 List of AnchorChip™ Compatible Chemicals and Plastics

MALDI Matrices

We recommend to use Brukers dedicated purified MALDI matrices.

3-Hydroxypicolinic acid (3-HPA)	1 g #201224,	5 g #203070
α-Cyano-4-hydroxycinnamic acid (HCCA)	1 g #201344,	5 g #203072
Sinapinic acid (SA)	1 g #201345,	5 g #203073
2,5-Dihydroxybenzoic acid (2,5-DHB)	1 g #201346,	5 g #203074
SDHB ("super DHB")		5 g, #209813

Solvents

HPLC grade or better

Plastics

Many plastics are not compatible with AnchorChip™ because they release polymers.
Avoid all kind of siliconized tubes!
Problems with unsuitable plastics typically result in strange looking crystallization and polymer signals in the spectra.
In extreme cases samples do not crystallize at all and remain liquid even after hours.
The following brands have been proven to be compatible with AnchorChip™.

Pipette tips	Eppendorf standard tips	http://www.eppendorf.com
Vials / tubes	Eppendorf safe-lock	http://www.eppendorf.com
Bottles	Nalgene FEP (Teflon) bottles	http://nalgenelab.nalgenunc.com
MTPs	Greiner PP, natural, MTP	http://www.greinerbioone.com

ZipTips™

AnchorChip™ technology is compatible with C18 ZipTip™ sample purification (http://millipore.com), provided the organic solvent concentration in the elution buffer does not exceed >50 %, otherwise you may get polymer peaks (from bed material!)




10 Troubleshooting

Problem	Reason	What to do
Strong polymer signals show up in mass spectra.	Most likely wrong plastics or analyte pretreatment.	Strictly follow rules given in paragraph 4.1.
Sample does not dry even after hours.	Surface active contaminant which blocks the evaporation. Most likely wrong plastics, buffers or analyte pretreatment.	Strictly follow rules given in paragraph 4.1. Wash target again (paragraph 3)!
Crystallization looks “ill”.	Buffers, salts, contaminants	Strictly follow rules given in paragraph 4.1. Try washing / recrystallization. Try ZipTip™.
Preparation exceeds anchor with water soluble matrix (2,5-DHB, 3-HPA)	Too much matrix.	Reduce molar amount of matrix.
Preparation exceeds anchor with water insoluble matrix (HCCA, SA)	Too much water.	Try recrystallization with an appropriate solvent. (paragraph 4.4.1, 4.5)
Laser hits many anchors off-center after teaching.	Teaching positions not optimal.	Edit the respective geometry file and replace the corner teaching positions (A1, A24, P1, P24) by inner positions (C3, C22, N3, N22).

11 Literature

- M. Schuerenberg, C. Luebbert, H. Eickhoff, M. Kalkum, H. Lehrach, E. Nordhoff
  Prestructured MALDI-MS Sample Supports
  Anal. Chem. 72 (2000) 3436 – 3442
- J. Gobom, M. Schuerenberg, M. Mueller, D. Theiss, H. Lehrach, E. Nordhoff
  α-Cyano-4-hydroxycinnamic Acid Affinity Sample Preparation.
  A Protocol for MALDI-MS Peptide Analysis in Proteomics
  Anal. Chem. 73 (2001) 434-438

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