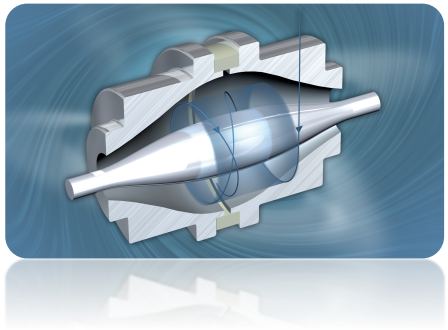
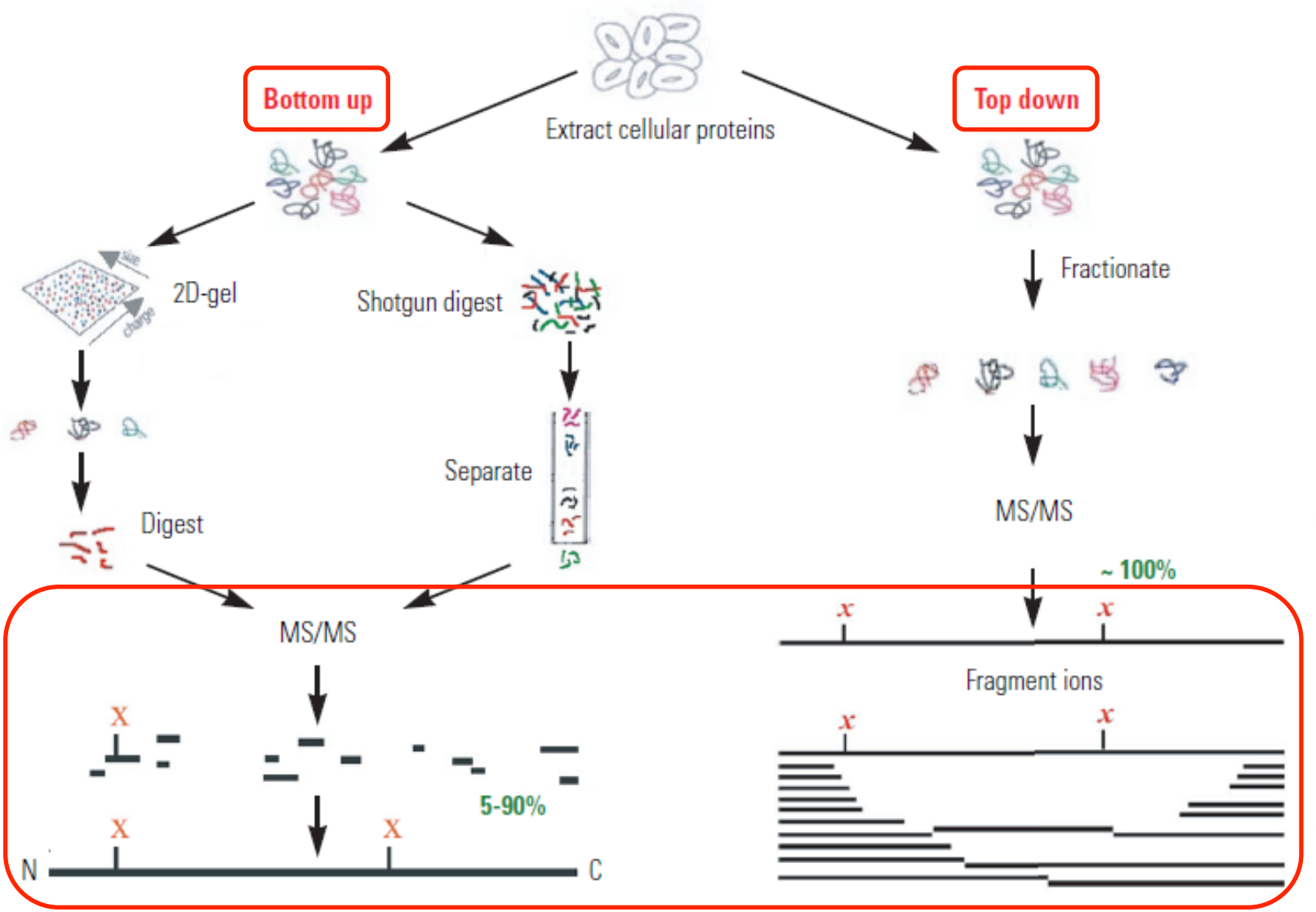


LTQ-Orbitrap-ETD质谱的应用

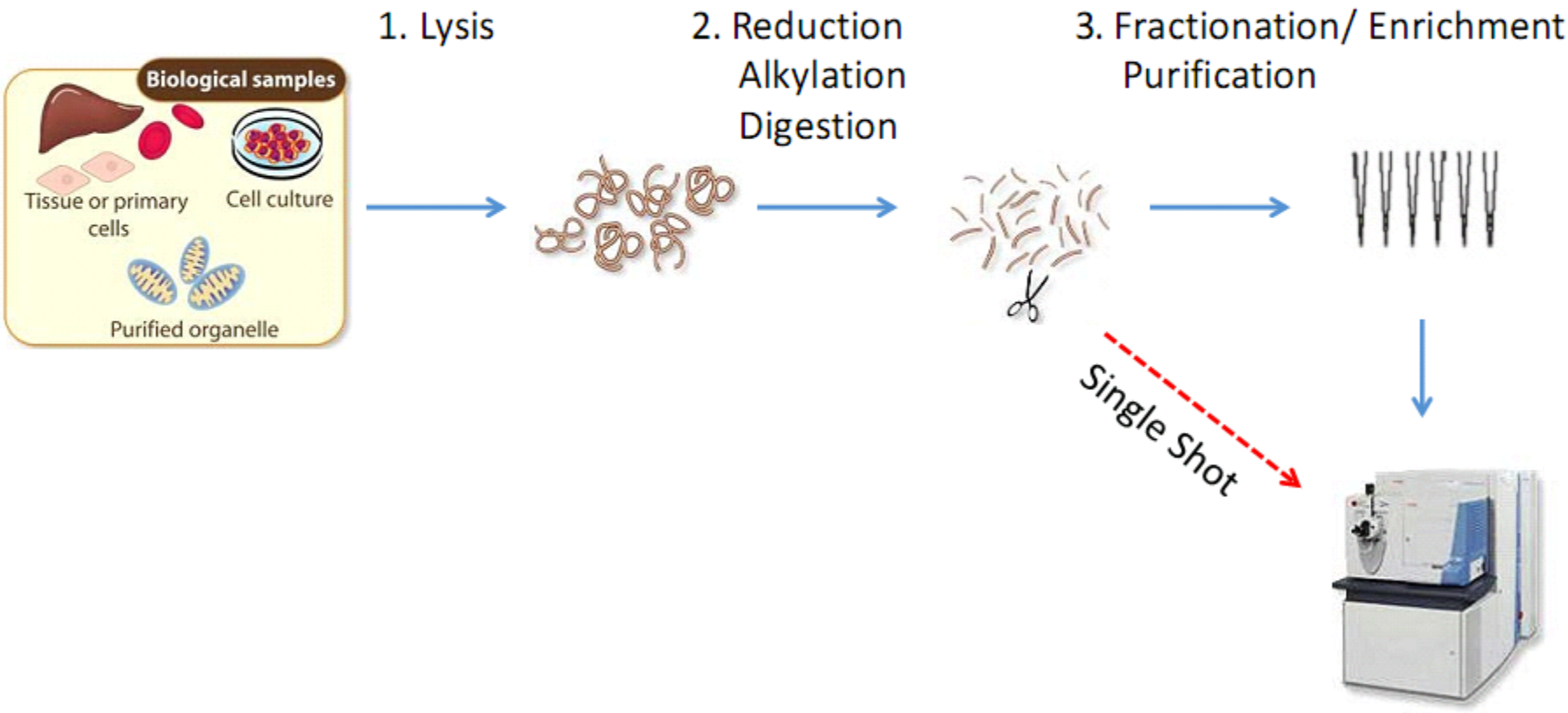
李 征

2014.03.17

蛋白质检测分析流程


Extract cellular proteins
Bottom up
Top down
2D-gel
Shotgun digest
Fractionate
Separate
Digest
MS/MS
~ 100%
MS/MS
Fragment ions
5-90%
N
C
蛋白质提取
蛋白质酶解与分离
质谱鉴定

From Biological Sample to MS Analysis



Digestion Protocols

In gel

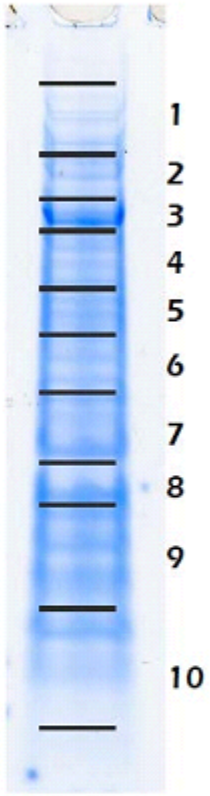
In Solution

FASP

Digestion Protocols

- **'In gel' protocol**

- Separation of proteins by SDS-PAGE (up to 100 µg)
- Staining with Coomassie Brilliant Blue
- Divide gel lane into fractions
- Cut gel in small pieces (~1 mm3)
- Washing of stain and SDS
- Dehydration
- Rehydration with Trypsin and incubation over-night
- Elution of peptides from gel pieces



Digestion Protocols

- **'In solution' protocol**

- Protein precipitation to eliminate detergents

- Re-solubilization with urea/thiourea (8M)

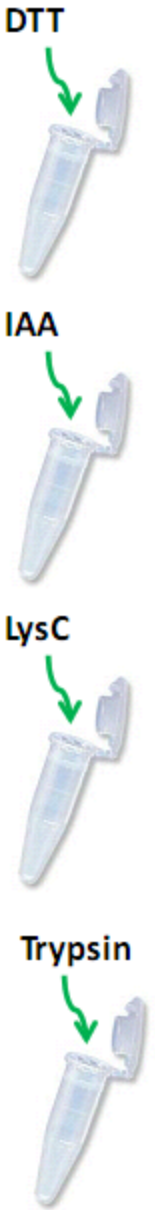
- Reduction with DTT

- Alkylation with iodoacetamide (IAA)

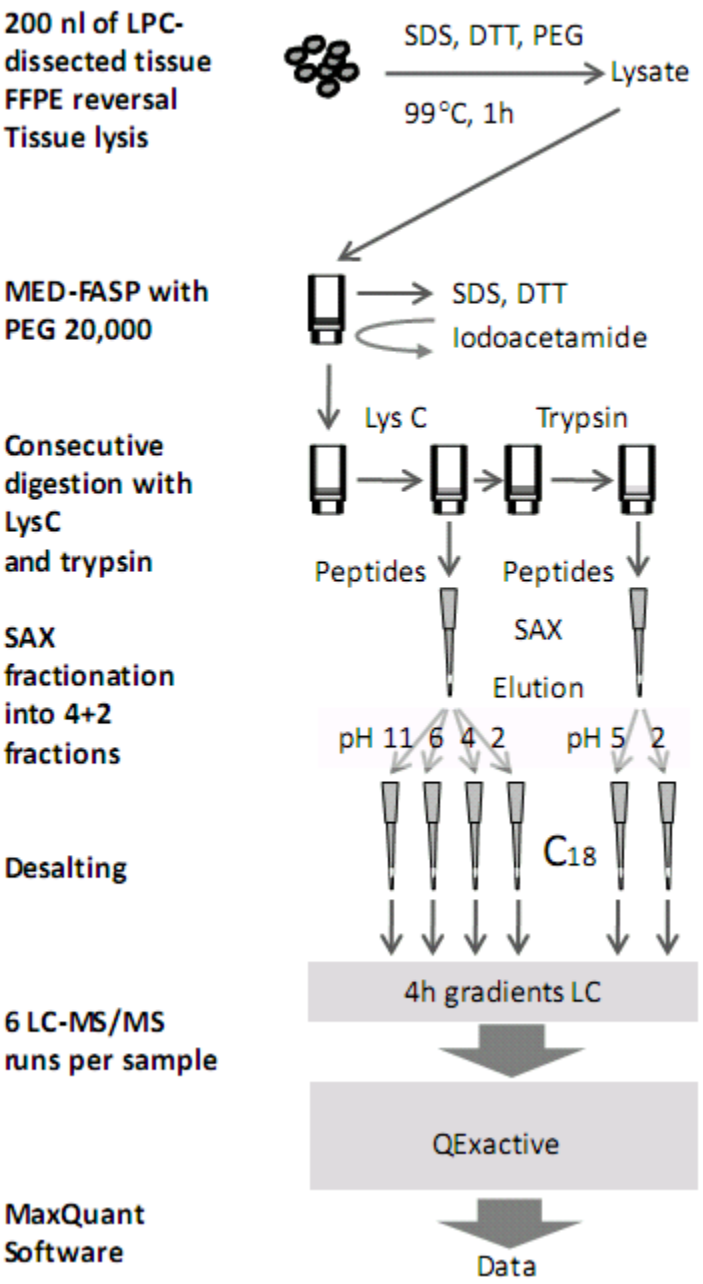
- LysC digestion

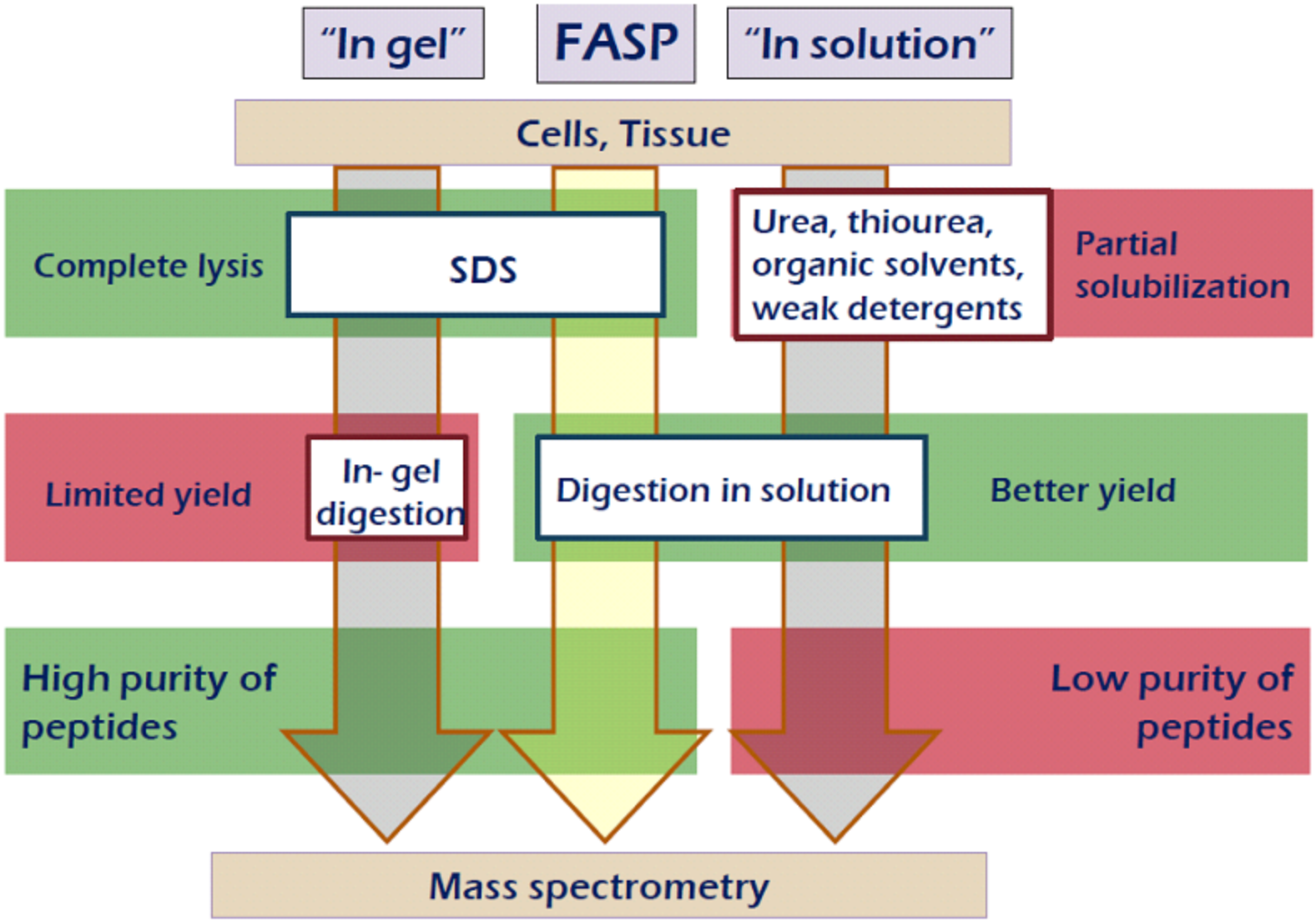
- Dilution of urea and incubation with trypsin over-night

- Trypsin inactivation with acid

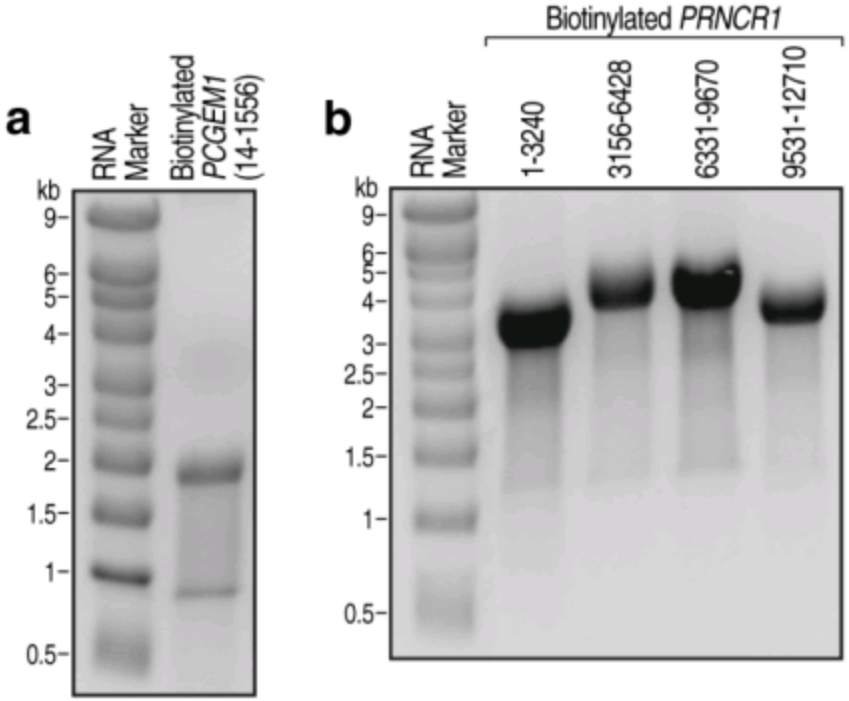


MED FASP for FFPE Samples




"In gel"
FASP
"In solution"
Cells, Tissue
Complete lysis
SDS
Urea, thiourea, organic solvents, weak detergents
Partial solubilization
Limited yield
In- gel digestion
Digestion in solution
Better yield
High purity of peptides
Low purity of peptides
Mass spectrometry

五、DNA 与蛋白相互作用



c

PRNCR1 and *PCGEM1* Associated Transcriptional Factors/Coactivators

Protein	*Peptides*
Monoavidin Beads only	
protease, serine, 1 preproprotein	1
PREDICTED: hypothetical protein	1
Biotinylated *PRNCR1* LncRNA Pulldown	
DOT1-like, histone H3 methyltransferase	7
coactivator-associated arginine methyltransferase	4
growth arrest and DNA-damage-inducible, alpha	5
androgen receptor isoform 1	3
Biotinylated *PCGEM1* ncRNA Pulldown	
catenin (cadherin-associated protein), beta 1, 88kDa	7
androgen receptor isoform 1	6
pygopus homolog 2	7

lncRNAs PRNCR1 和PCGEM1在侵袭性前列腺癌中高表达,明确PRNCR1 和PCGEM1与雄激素受体AR构成复合物，影响下游转录与翻译。

lncRNA-dependent mechanisms of androgen-receptor-regulated gene activation programs NATURE 2013

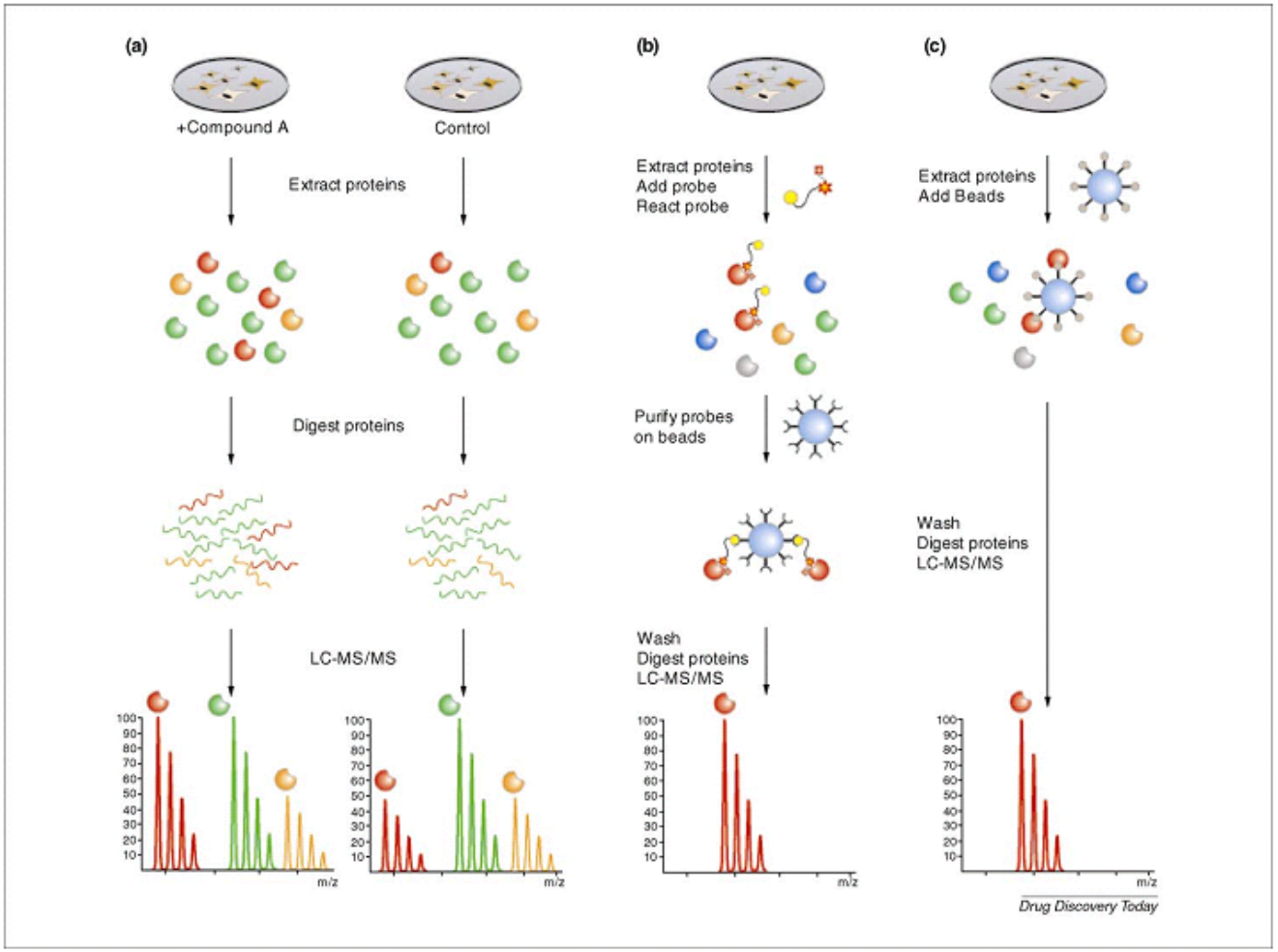
五、DNA 与蛋白相互作用

e

Residue	Conf	Sequence	Prec M/Z	Expe M/Z	Modifications
K143	0.065	AASKGLPQQL	1012.17	1105.62	Phospho(S)@3; Methyl(K)@4
K237	46.54	DNYLGGTSTISDNAKE	1684.74	1708.83	Dehydrated(D)@12; Deamidated(N)@13; Acetyl(K)@15
K291	2.446	KGSLLDDS	833.89	927.43	Methyl(K)@1; Phospho(S)@3
K318	0.05	TKGLEGESL	933.03	1026.56	Methyl(K)@2; Phospho(S)@8
K349	0.57	LSLYKSGALDE	1195.34	1288.59	Methyl(K)@5; Phospho(S)@6
K631	19.29	KLKKLGNLKLQEEGEA	1798.1	1854.86	Acetyl(K)@1; Oxidation(K)@9
K634	0.05	LGARKLKKL	1026.32	1083.64	Oxidation(K)@5; Acetyl(K)@8

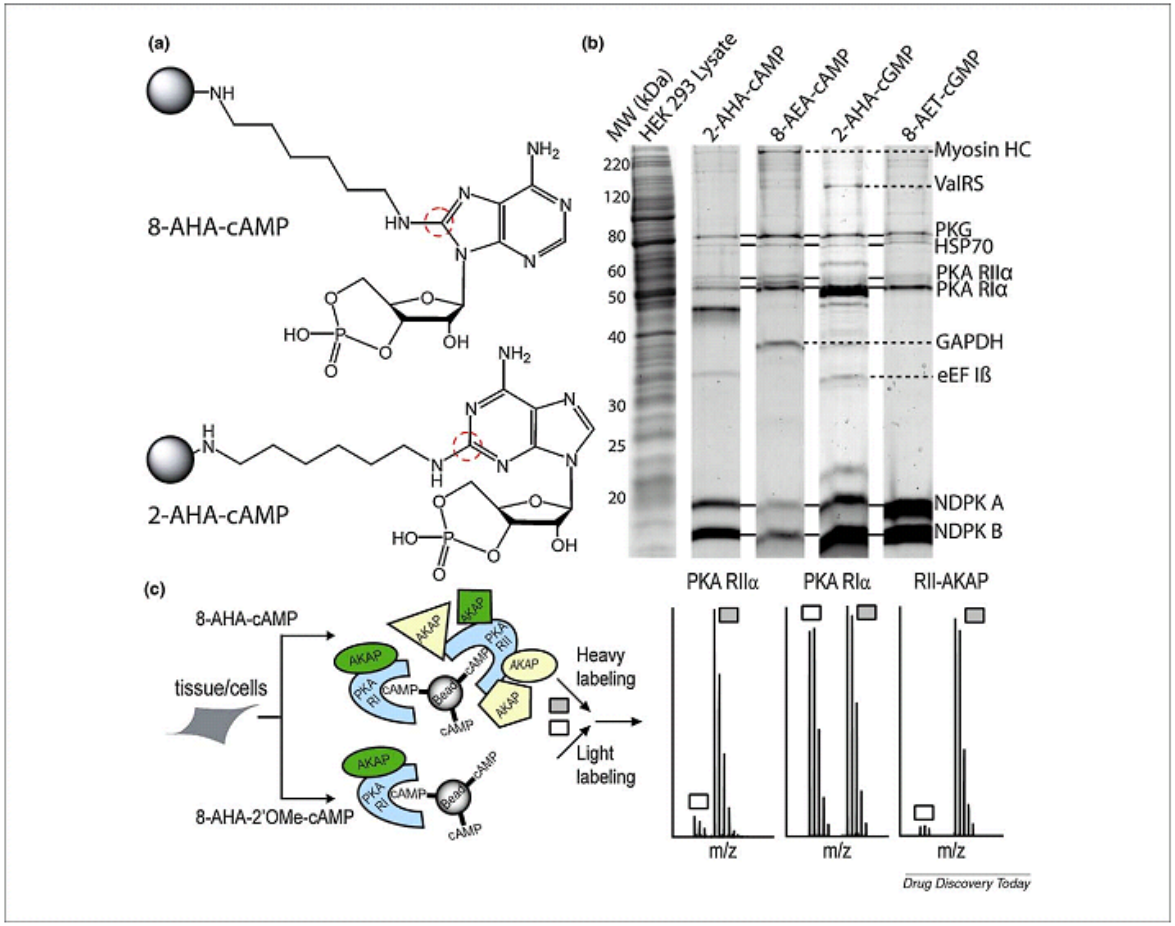
lncRNA-dependent mechanisms of androgen-receptor-regulated gene activation programs NATURE 2013

六、寻找药物作用靶标



Drug Discovery Today Volume 14,Numbers 21/22 November 2009

六、寻找药物作用靶标



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