Computational Systems Biology
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Lecture - 83
13C-Metabolic Flux Analysis using Mass Spectrometry

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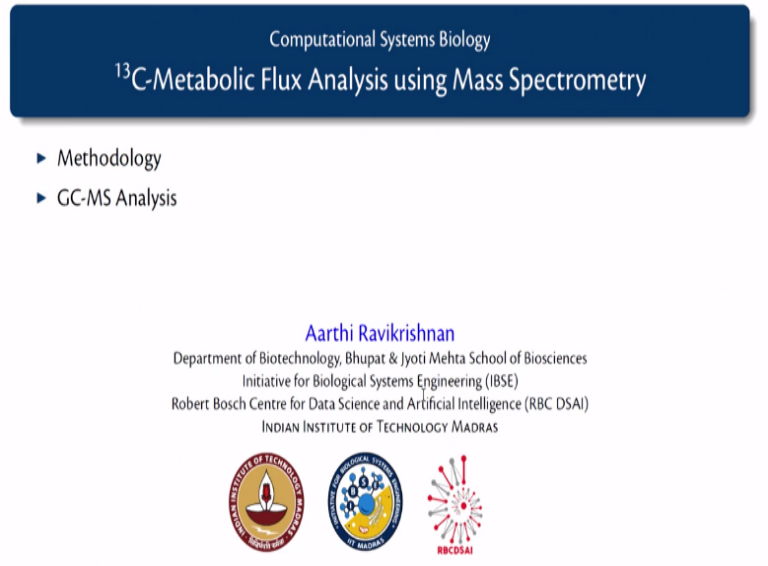

In this video, we will be looking at the experimental methodologies to carry out metabolic flux analysis and also look at GC-MS data using labeled amino acids to understand how we can carry out 13C flux analysis using mass spectrometry.

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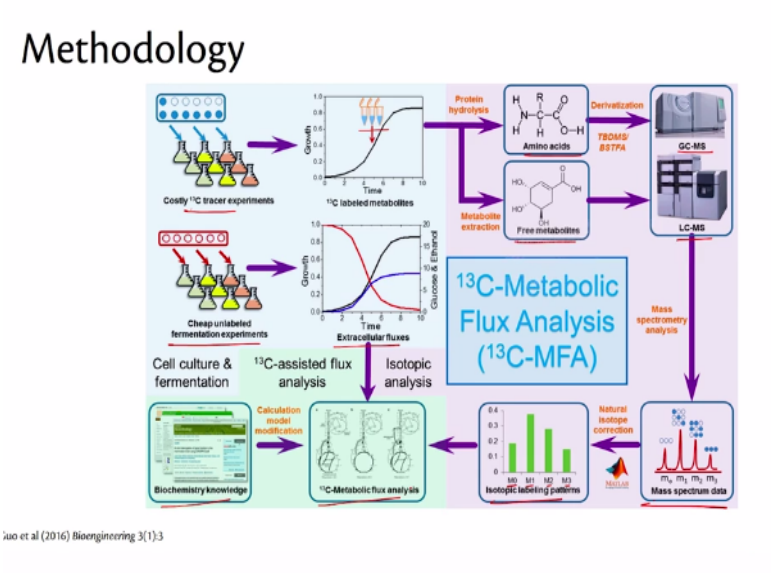

So this is the overall methodology for carrying out C13 metabolic flux analysis. So first you start with cheap unlabelled fermentation experiments where you try to assess the sub state uptake rate, the product formation rate and so on and also calculate the growth curve because the time at which you take your samples for carrying out further analysis is heavily dependent on the phase at which the bacterial cells grow, bacterial or E cells or any cells grow.

So you need to first understand the physiology of the cells and also understand the different rates at which the product is produced. The substrate is utilized and so on and from this you then go to the costly C13 tracer experiments where you again do the same kind of analysis and you take out samples at different time points or at a particular time point depending on the type of experiment you do and from that you take the biomass of the cells and find out the amino acid labeling.

I will come to why you want to use on the amino acids in the next few slides. And also see the free metabolites so you basically take the biomass, hydrolyze it, get the amino acids, do further processing by GCMS about which we will come to in the next slides and also analyze the free metabolites through other techniques like LCMS and then find out to find out the labeling patterns.

And using this labeling pattern, so you identify the mass spectrum, you get the mass spectrum and you find out the isotropic labeling patterns so here you will have data for M0, M1, M2, and 3

which I earlier showed you as M, M + 1, M + 2, M + 3 and you fit it into a model which has these equations which can tell you about the relative flux ratios or you know the flux through the particular path way and so and this model is a small model generally they start with the small model.

They do not go with the larger genome scale models simply because it gets too large for the data to fit and this core model can be built from the biochemistry knowledge or from different databases just take the core network and fit all the fluxes and also you take the extra cellular fluxes from the earlier experiments fitted into the model and then generate something called a metabolic flux map and this map can tell you about the fluxes through different pathways.

So this is the overall methodology which is followed in carrying out 13C metabolic flux analysis.

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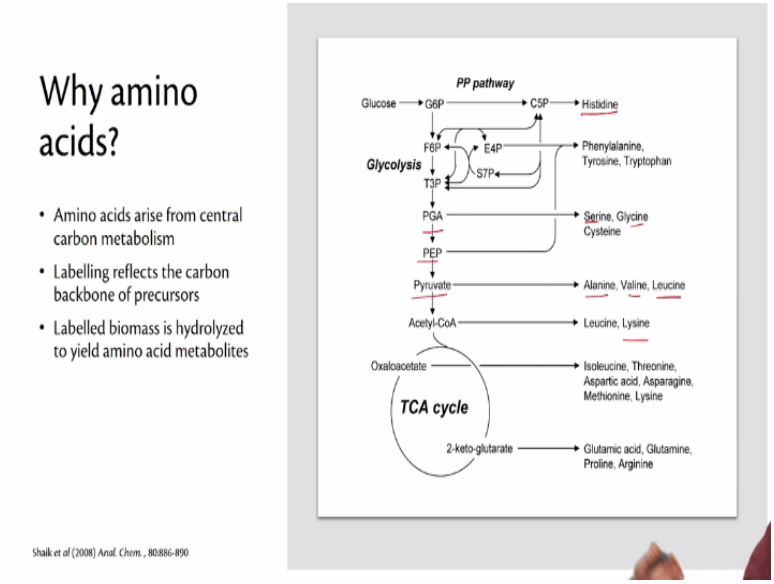

So while I was talking about taking samples and analyzing the biomass I was very specific about amino acids. So why should I talk about amino acids. So this is because these amino acids arise from central carbon metabolism and when you take these samples like say histidine, serine, glycine all these are a part of central carbon metabolism and when you find out the labeling patterns of these amino acids they serve as a proxy for identifying the fluxes of different central carbon metabolites.

So something like if you want to find out the flux through PEP the action from PEP to pyruvate you might want to look at the labeling patterns of alanine, valine, leucine and then infer the rates at which pyruvate was produced. So this is why amino acids are most preferable and also the biomass composition, the biomass is composed of many amino acids and these amino acids have their own set of advantages while doing experiments which I will tell you in the next slides.

And also the label biomass I mean so you basically take the label biomass and hydrolyze them to get the amino acid metabolites.

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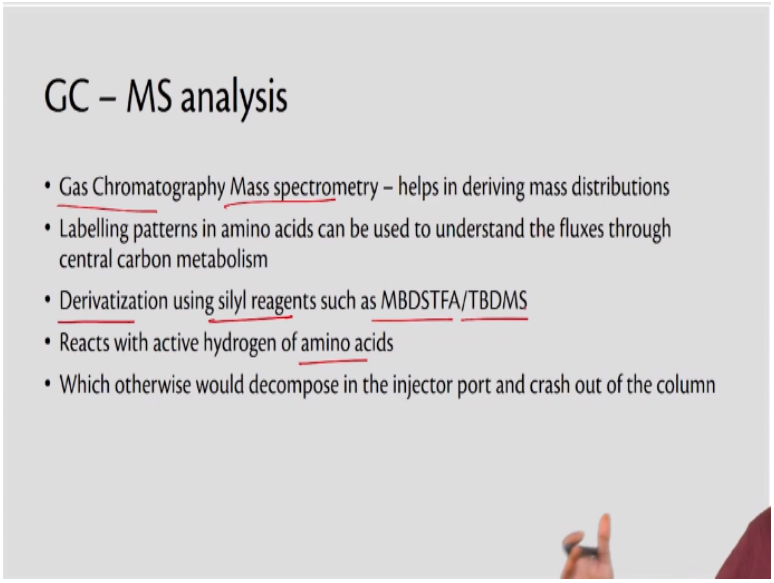

So, now talking about the GC-MS analysis which forms the basic part of any metabolic flux analysis. So this is basically a technique to identify the mass distributions. So GC-MS is simply a technique where you have gas chromatography couple with mass spectrometry and you analyze the labeling patterns in different amino acids to understand the flux using this particular technique and if you want to do GC-MS analysis the substance that you might want to analyze.

Say for example amino acid has to be volatile because it is going to be subjected to high temperature and it has to be volatile and we find that the amino acids are not volatile in nature, but we make them volatile by derivatizing using silyl reagents such as MBDSTFA the name is very big. This is again a silyl reagent and or TBDMS and using these we get a derived form of

amino acid which when subjected to GC-MS analysis would give you different fragments and using these fragments you try to identify the labeling patterns.

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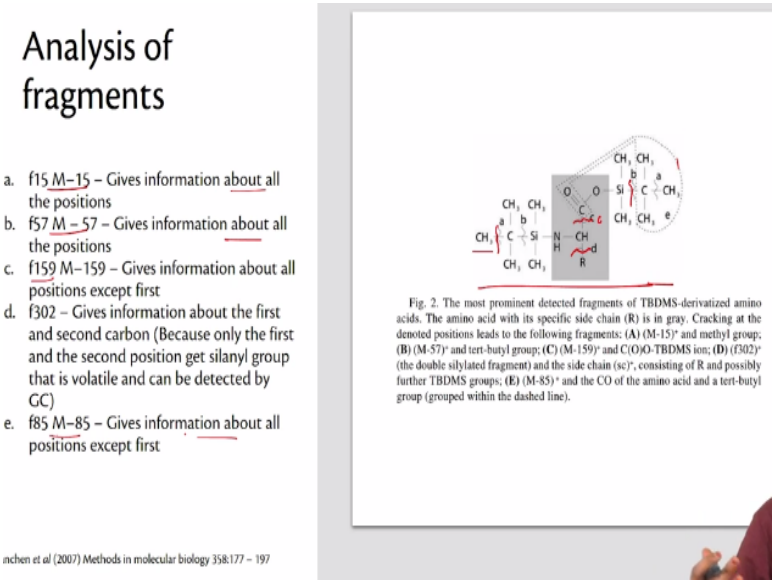

So this is the derived form of any amino acid so you have the silyl derivates which attack the hydrogen that is the reactive hydrogen of amino acid and form a derivative which when subjected to GC-MS analysis would lead to different fragments. So for instants if you subject this particular substance to GC-MS analysis the most prominent fragments that you observe are 5. So the first one, which I call f15 is M - 15.

So it gets fragmented at this position that is a so your methyl groups get cleaved. So the mass of the amino acid that you get would be M - 15 when you observe this particular fragment. So this is M - 15, 15 because the molecular weight of CH3 is 15. Similarly, if you subjected to say B, I mean if it gets fragmented at B you get f57 so the molecular weight of this particular group is 57 and so you get M - 57 and similarly if you do it at C. C is basically this point is C.

And this will tell you that it is M - 159 because the mass of the fragment that you get is - 159 and similarly there is another fragment that is F302 that is a double silylated fragment so that is coming out here and E is F - 85. So E is this, the 1 in the dots so that is M - 85 and each of these fragments give information about different positions of the amino acid. For instance, if you were to take a 3 carbon amino acid.

And if you were to analyze M -15 fragment it gives you information about all the 3 positions and if you take again M -57 it is going to give you information about all the 3 positions whereas when you take F159 it gives you information about all the positions except the first simply because the first gets cleaved and it is not detected by the GC-MS detector and similarly f85 that is M - 85 it gives you all information about all the positions except the first.

So using these information of different fragments you tend to part is or you tend to calculate the labeling at different positions.

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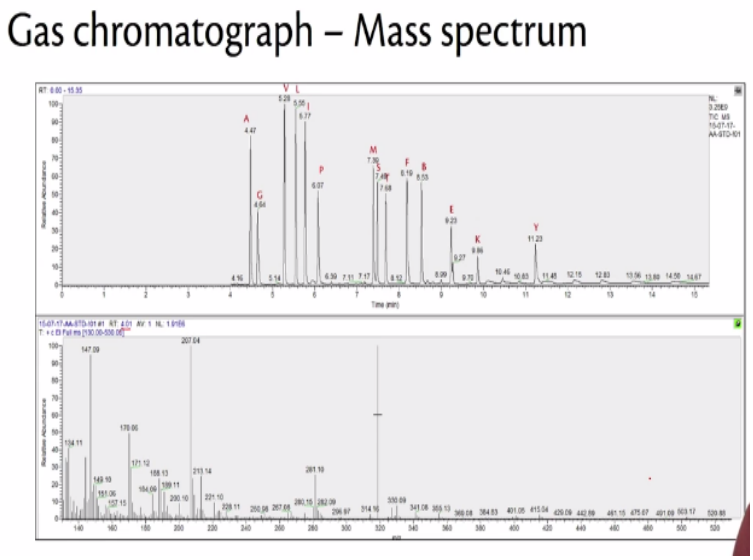

So this is how a gas chromatograph looks like I mean gas chromatograph mass spectrum looks like. So the first the top panel shows the retention times of different amino acids. So here you have this the order in which every amino acids gets eluted so you have alanine, valine, leucine, and so on and so forth and then in the bottom panel you can find the mass spectrum so for every retention time you calculate the ions.

You calculate the mass of different ions that get eluted and based on different mass or so here are you know you can find 207, 213, 221, and they are at different relative abundances and that every elusion times so here the time is 4.01 so in the retention time of 4.01 you can find all these ions. So this is how general gas chromatograph looks like.

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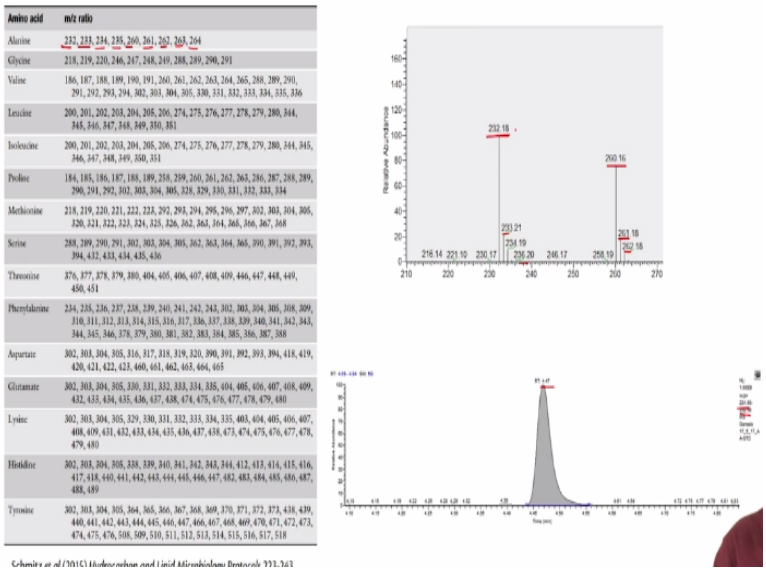

Amino acid	m/z ratio
Alanine	232, 233, 234, 235, 260, 261, 262, 263, 264
Glycine	218, 219, 220, 246, 247, 248, 249, 288, 289, 290, 291
Valine	186, 187, 188, 189, 190, 191, 260, 261, 262, 263, 264, 265, 288, 289, 290, 291, 292, 293, 294, 302, 303, 304, 305, 330, 331, 332, 333, 334, 335, 336
Leucine	200, 201, 202, 203, 204, 205, 206, 274, 275, 276, 277, 278, 279, 280, 344, 345, 346, 347, 348, 349, 350, 351
Isoleucine	200, 201, 202, 203, 204, 205, 206, 274, 275, 276, 277, 278, 279, 280, 344, 345, 346, 347, 348, 349, 350, 351
Proline	184, 185, 186, 187, 188, 189, 258, 259, 260, 261, 262, 263, 286, 287, 288, 289, 290, 291, 292, 302, 303, 304, 305, 328, 329, 330, 331, 332, 333, 334
Methionine	218, 219, 220, 221, 222, 223, 292, 293, 294, 295, 296, 297, 302, 303, 304, 305, 320, 321, 322, 323, 324, 325, 326, 362, 363, 364, 365, 366, 367, 368
Serine	288, 289, 290, 291, 302, 303, 304, 305, 362, 363, 364, 365, 390, 391, 392, 393, 394, 432, 433, 434, 435, 436
Threonine	376, 377, 378, 379, 380, 404, 405, 406, 407, 408, 409, 446, 447, 448, 449, 450, 451
Phenylalanine	234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 302, 303, 304, 305, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388
Aspartate	302, 303, 304, 305, 316, 317, 318, 319, 320, 390, 391, 392, 393, 394, 418, 419, 420, 421, 422, 423, 460, 461, 462, 463, 464, 465
Glutamate	302, 303, 304, 305, 330, 331, 332, 333, 334, 335, 404, 405, 406, 407, 408, 409, 432, 433, 434, 435, 436, 437, 438, 474, 475, 476, 477, 478, 479, 480
Lysine	302, 303, 304, 305, 329, 330, 331, 332, 333, 334, 335, 403, 404, 405, 406, 407, 408, 409, 431, 432, 433, 434, 435, 436, 437, 438, 473, 474, 475, 476, 477, 478, 479, 480
Histidine	302, 303, 304, 305, 338, 339, 340, 341, 342, 343, 344, 412, 413, 414, 415, 416, 417, 418, 440, 441, 442, 443, 444, 445, 446, 447, 482, 483, 484, 485, 486, 487, 488, 489
Tyrosine	302, 303, 304, 305, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518

Schmitz et al (2015) Hydrocarbon and Lipid Microbiology Protocols 333-363

And using this if you were to look at alanine which I was talking to you before the amino acid so you have different masses, you have derivatized masses. So these are the different M/Z ratios which you might observe for different amino acids for example if you have alanine it has 232, 233, 34, 35 and here again. So this is how your mass spectrum will look like. So you have 232, 33, 34. 35 is not here because it is not labeled and you also have other fragments which are being detected and these have the mass is starting from 260 to 264.

And where you can find the spectrum mass spectrum which consists of masses between 260, 261, and 62 and using this you tend to calculate the area under the curve for a particular retention time say at 4.47 I know that alanine is being eluted. I take the area under this curve for a particular M/Z ratio say for example 231.5 to 232.5 so this would give an idea of what how many ions came out at time 4.47 whose masses are between 231 and 32 and this would give me an idea of this particular M/Z ratio.

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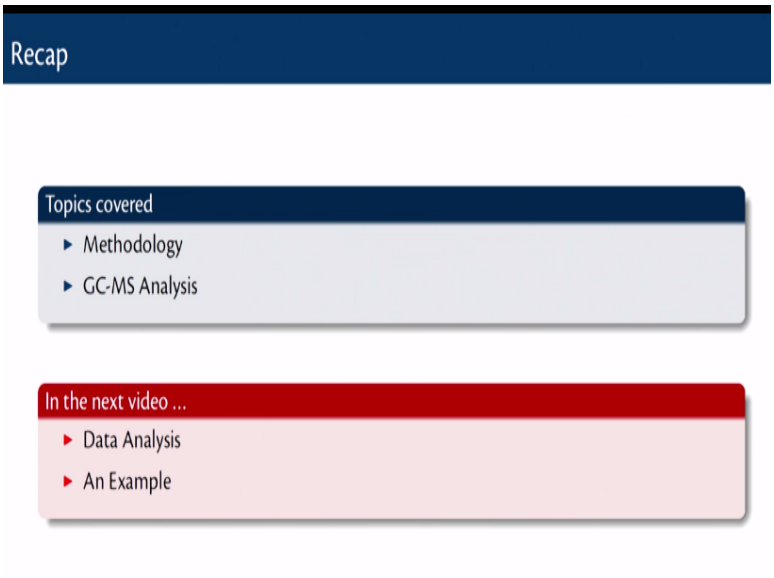

In this lecture we looked at the methodology to understand how to carry out C13 metabolic flux analysis and also had an idea of how the data from GC-MS analytical technique looks like and how the labeled amino acids will show up on GC-Ms chromatogram. In the next video we will look at how to use this data and perform metabolic flux analysis and also discuss a small example where 13C metabolic flux analysis has been carried out.