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SMCR: A new approach to recovering temporal metabolic signal modulation in NMR spectroscopic datasets

Application to a life-long caloric restriction study in dogs

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Caloric Restriction

- Natural intervention
  - Increase longevity
  - Reduce onset and prevalence of late life diseases
  - McCay *et al.* 1930's
    - Yeast, worms, fruit flies, rodents and dogs
- Nestle Research Centre
  - CR in Labrador Retrievers
    - 1.8 years longer median lifespan
    - Osteoarthritis and neoplastic diseases
  - Biochemical and physiological process unknown
    - Retardation of cellular, DNA and macromolecular damage

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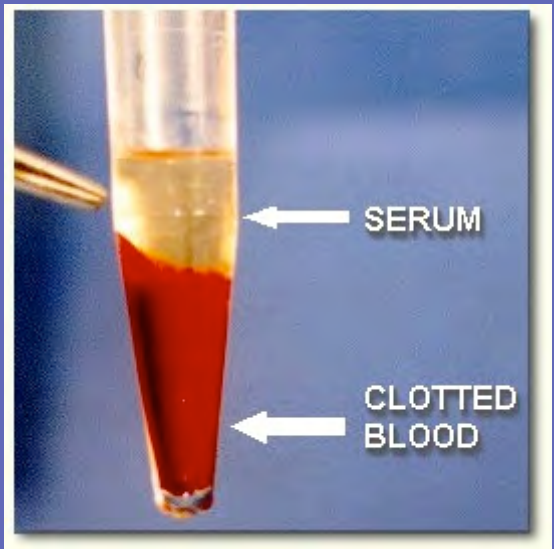
Dynamic biofluid

- Nutritional metabonomics (^{1}H NMR blood serum)
  - Distinctive biomarkers associated with dietary intervention
    - Dynamic concentration changes in lipids, lipoproteins and ketone bodies as system maintains homeostasis
    - Mask subtle systematic changes associated with diet



- Inter-subject variability is large
  - Severely complicates retrieval of statistically valid biomarkers
    - Variation in recency of dietary intervention and differences between individuals metabolic phenotype
    - Identify an individuals healthy state
- Advantages
  - Readily available & relatively non-invasively
  - Rich source of nutrients transit from one organ to another
    - Reflect current state of health and disease

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Objectives

New Information-rich model free metabonomics approach is introduced to characterise the temporal metabolic response of dogs to lifetime CR

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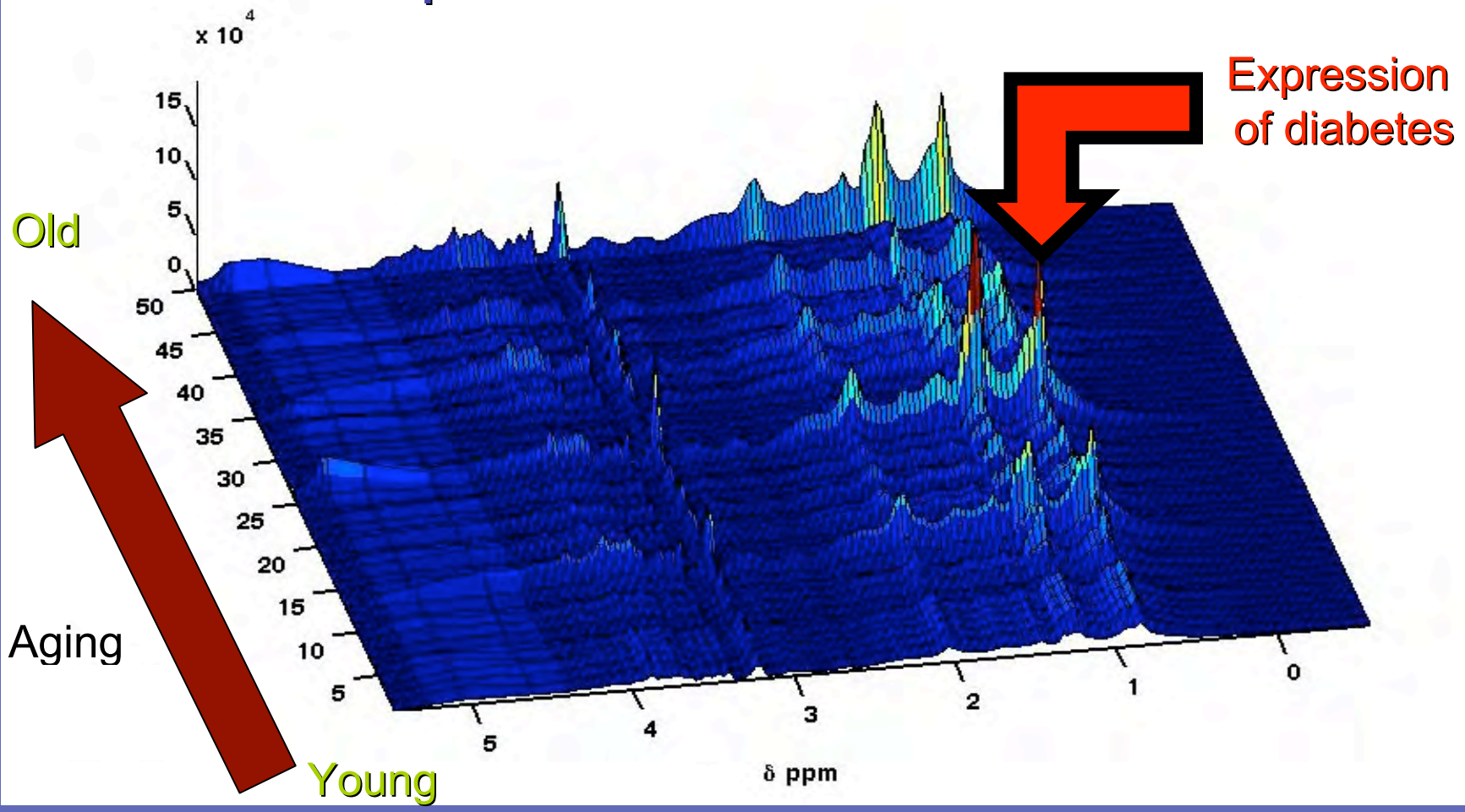
Objectives

^{1}H NMR metabolic profile



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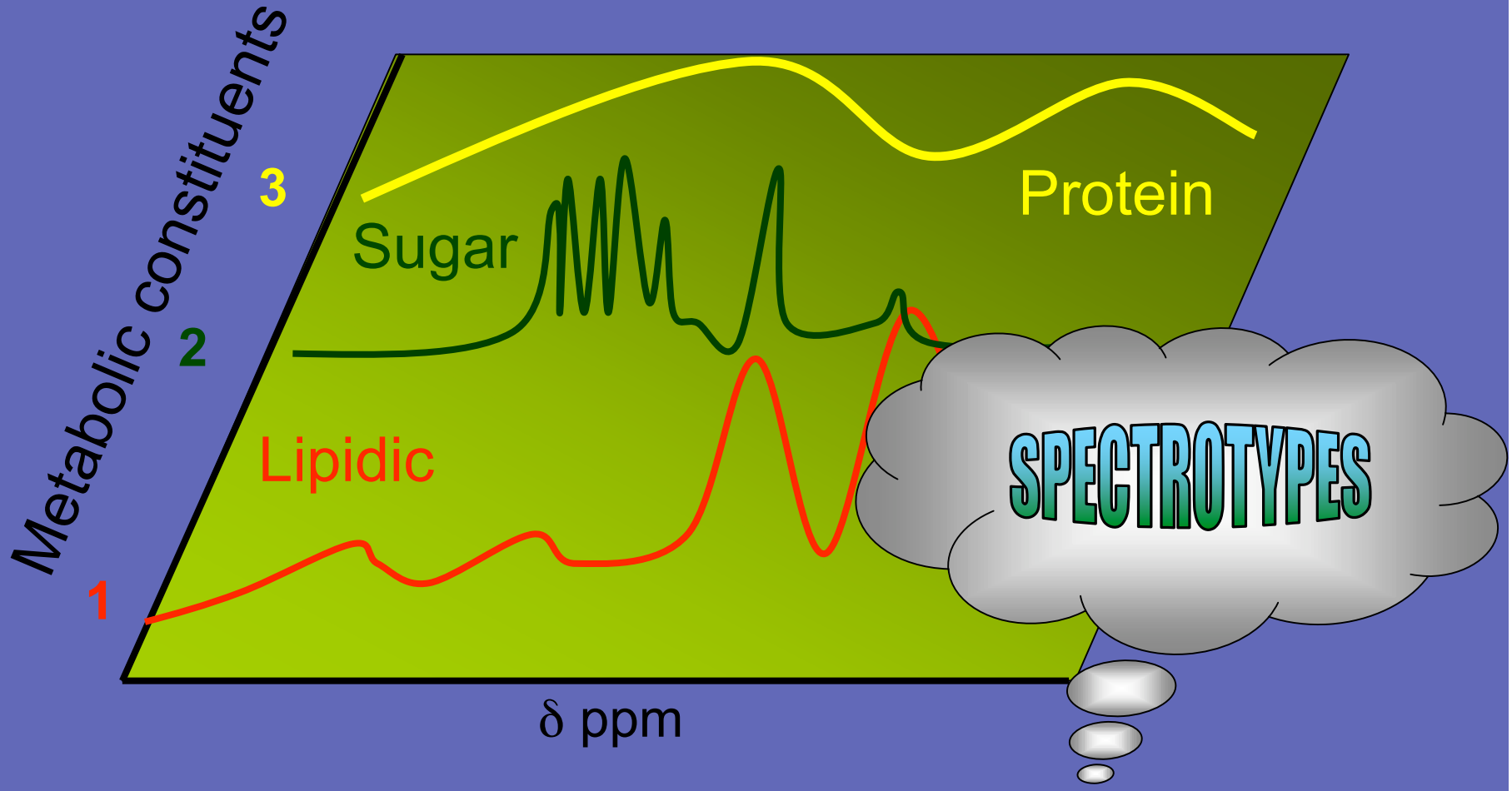

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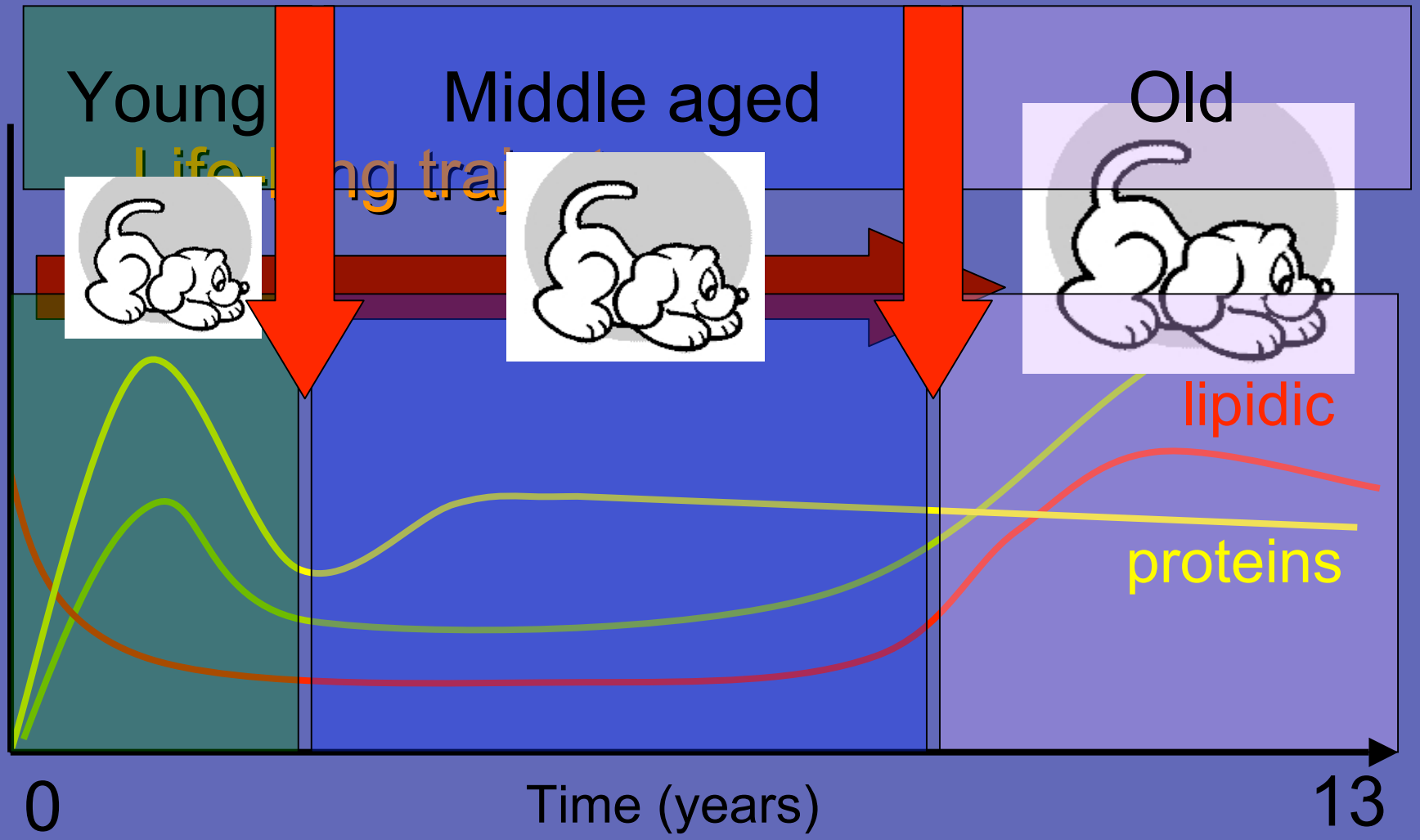

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Self Modeling Curve Resolution

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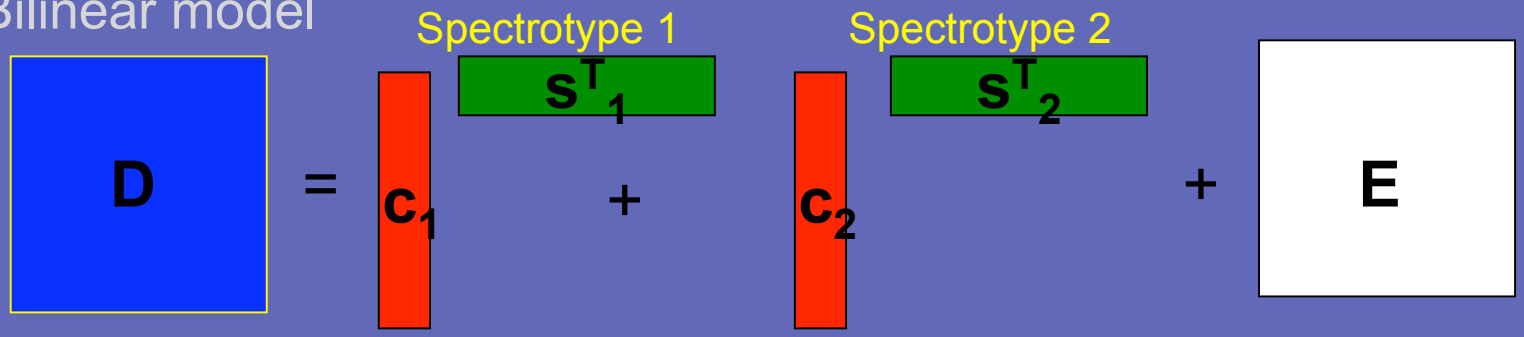
SMCR Theory

- Underlying Principle of SMCR
  - Bilinear model



- Initial Estimates
  - Quantitative Iterative Target Transformation Factor Analysis (QITTFA)
    - Starting estimates approximate the final solution
    - Advantages
      - Refinement of Initial estimates prior to ALS
      - Absence of unstructured variance
    - Five Steps of the QITTFA routine

$$E = ||D - C S^T||$$

Richards, S. E.; Walmsley, A. D. *Journal of Chemometrics* **2007**, *22*, 63-80.

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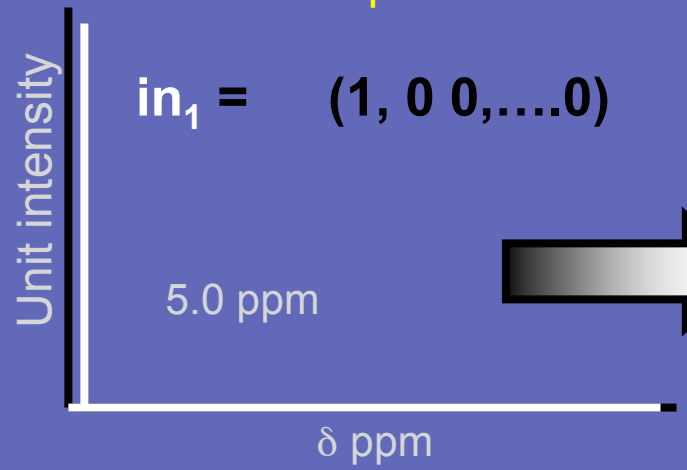
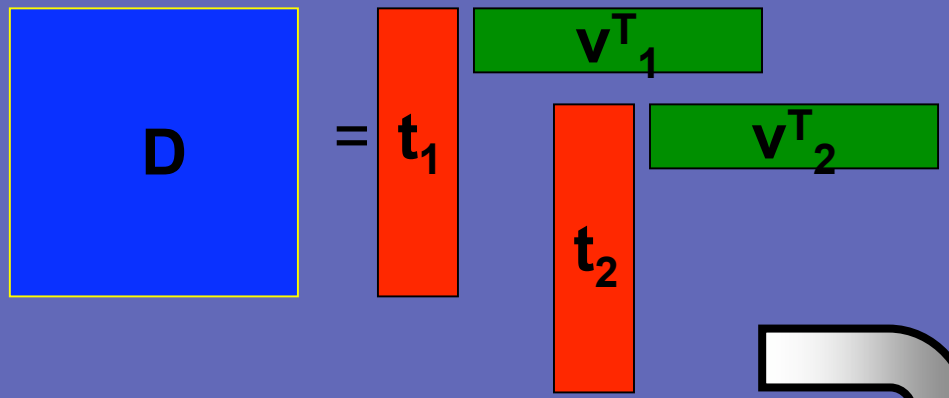
Initial Estimates (QITTFA)

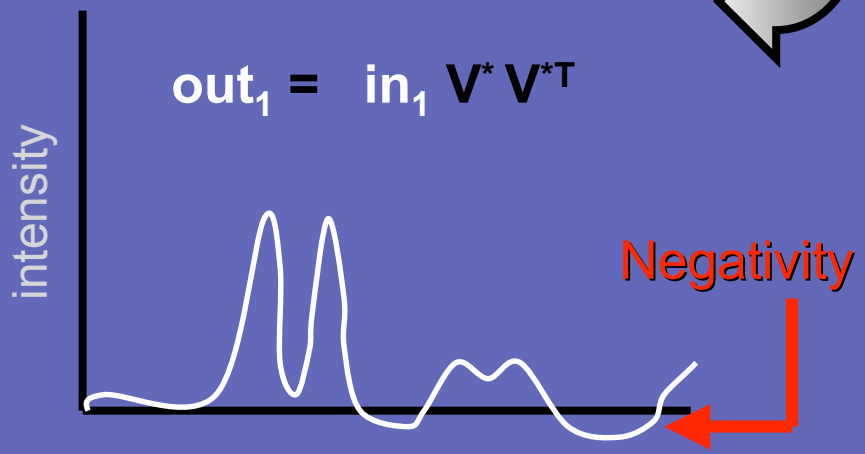
1. Needle Spectrum



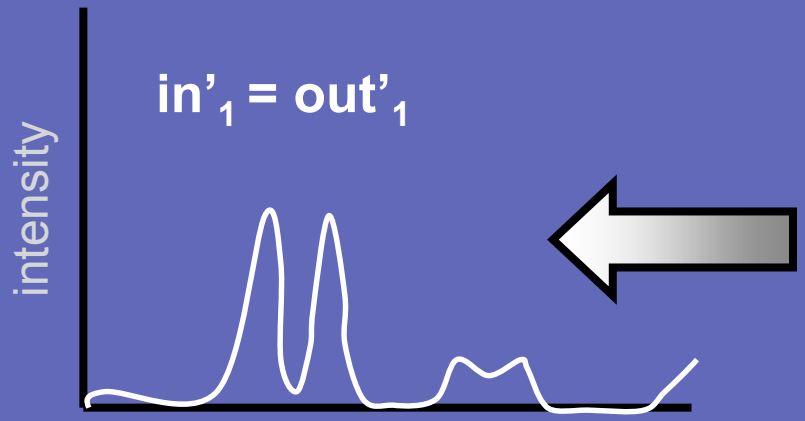
2. Singular Value Decomposition



3. Needle Output Spectra



4. Needle Output Spectra Constrained



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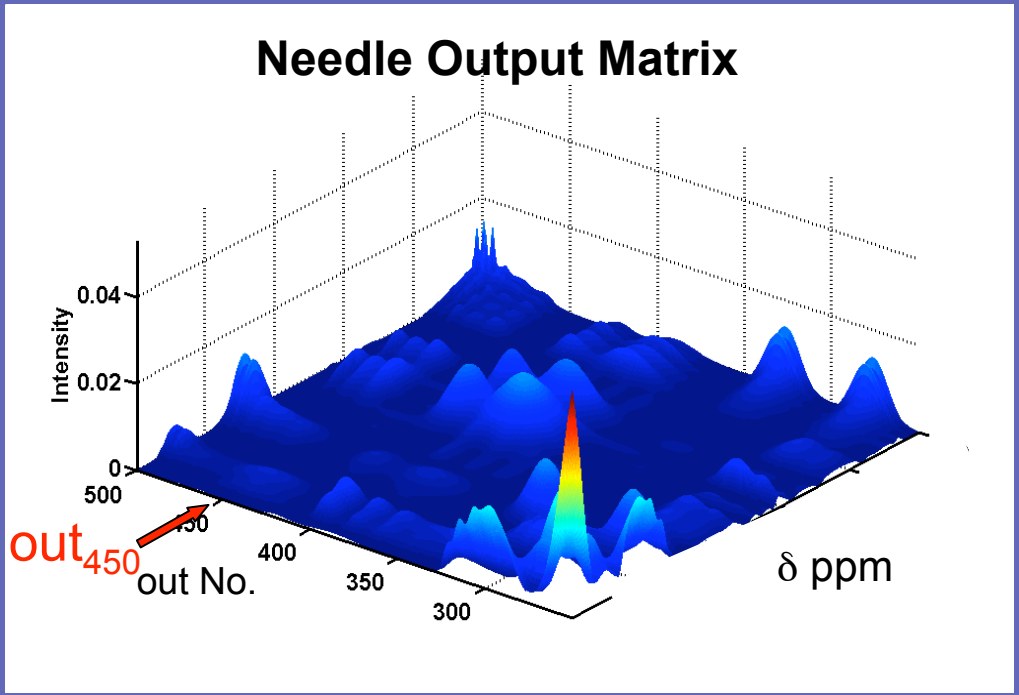
Initial Estimates (QITTFA)

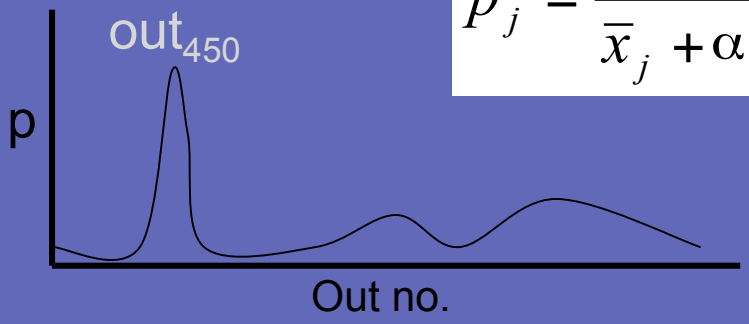


5. Selection of Output Spectra (Initial Estimates)

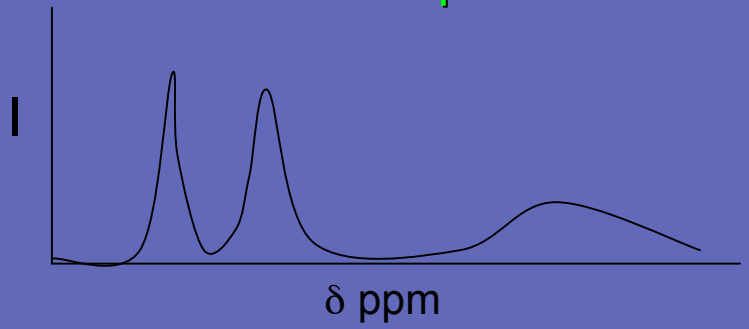
SIMPLISMA

Purity spectrum

$$p_j = \frac{s_j}{\bar{x}_j + \alpha}$$



Initial estimate spectrum No. 1



Which is the purest **out** (initial estimate)?

$out_1 = in_1 V^* V^{*T}$

$out_2 = in_2 V^* V^{*T}$

$out_m = in_m V^* V^{*T}$

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Validation of SMCR

Alternating Least Squares (ALS)

$$C = D\,(S^T)^+$$

Constrain?

Constrain?

$$S^T = C^+\,D$$

$$E = \|D - C\,S^T\|$$

Rotational Ambiguity

$$D = C\ S^T$$

$$D = (CT)\,(T^{-1}S^T)$$

Intensity Ambiguity

$$D = \sum_{i=1}^{n} (1/k_i c_i)\,(k_i s_i^T)$$

Lack of fit (LOF)

$$LOF(\%) = 100\sqrt{\frac{\sum_{ij}\left(d_{ij} - \hat{d}_{ij}\right)^2}{\sum_{ij} d_{ij}^2}}$$

% Variance explained

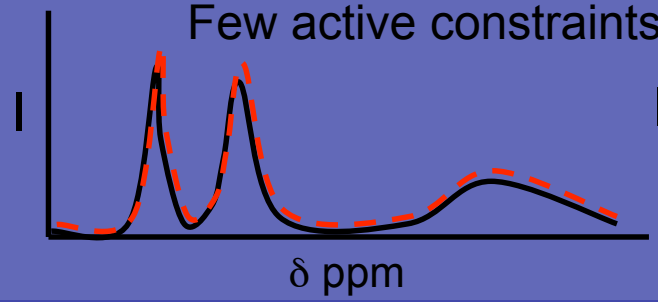
$$r^2(\%) = 100 \times \frac{\sum_{ij} \hat{d}_{ij}^2}{\sum_{ij} d_{ij}^2}$$

Constrained (---)

Unconstrained (- -)

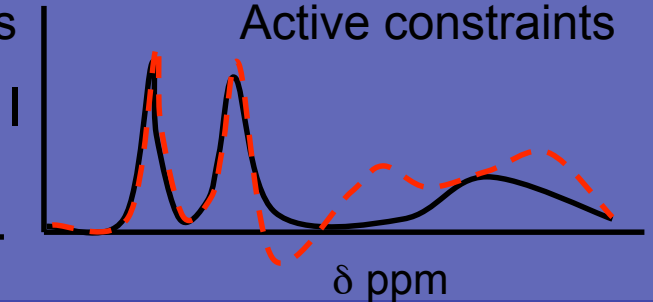
Good match

Few active constraints



Poor match

Active constraints



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Experimental

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Experimental Design & Analysis

- Animal Handling
  - 48 dogs
    - paired gender and weaning weight
    - Randomly assigned (CF/CR)
    - Initiated 8 weeks
    - CR (75% of CF)
- NMR spectroscopy
  - 400 µl of saline (10% D_2O) in 200 µl blood plasma
  - Bruker DRX 600 NMR spectrometer
    - 600.13 MHz for ^1H
    - NOESY
- Binned Data (0.005 ppm)

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Experimental Design & Analysis

- Multivariate Metabolic Trajectory
  - PCA Trajectory plots
    - Reveal patterns and trends in the data
    - Scaled to UV
  - SMCR Analysis
    - QITTFA Initial Estimates
      - Non-negativity constraints, maximum iteration 500
    - ALS
      - Non-negativity constraints in C and S and normalization of S

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Results and Discussion

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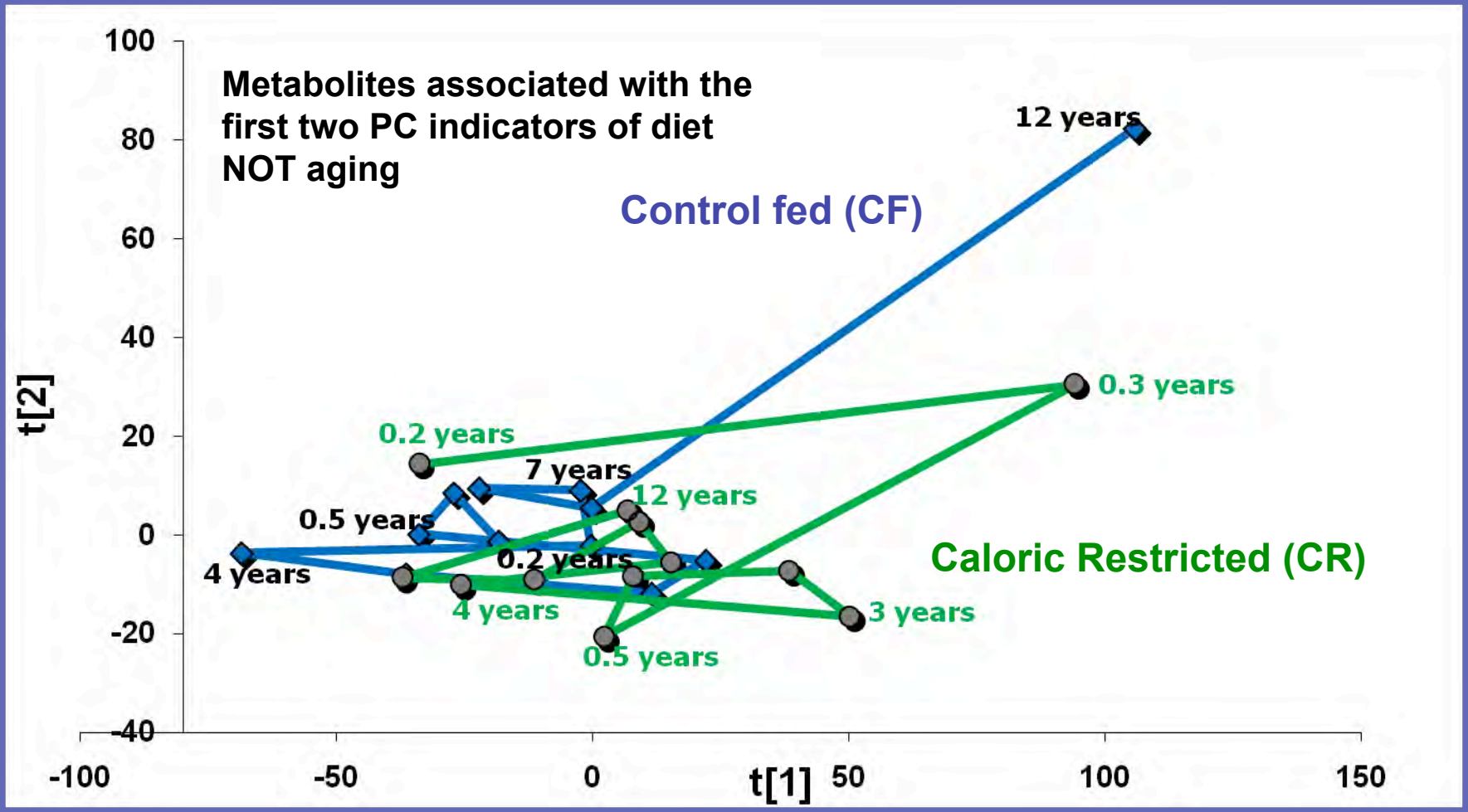
Multivariate Metabolic Trajectory (PCA)



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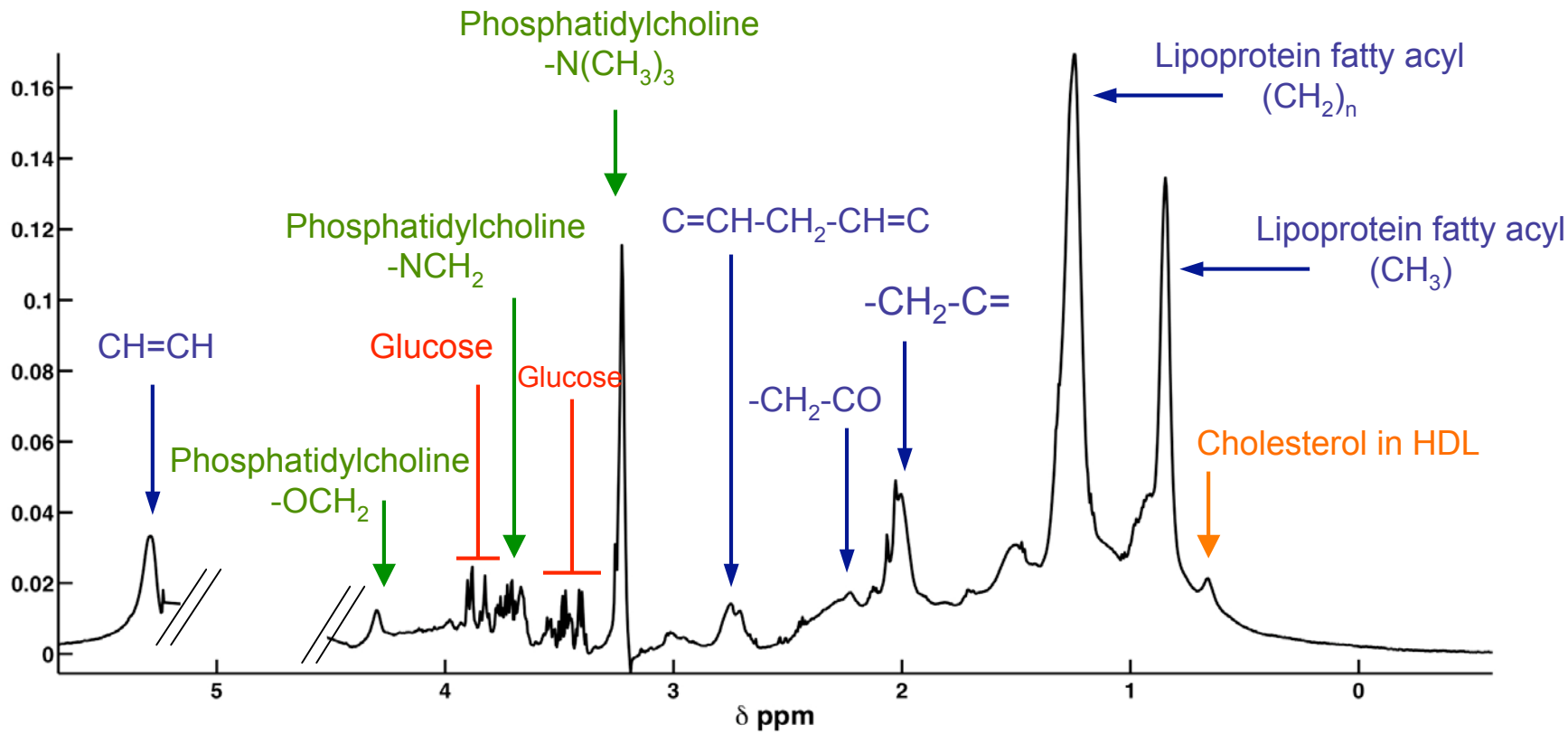
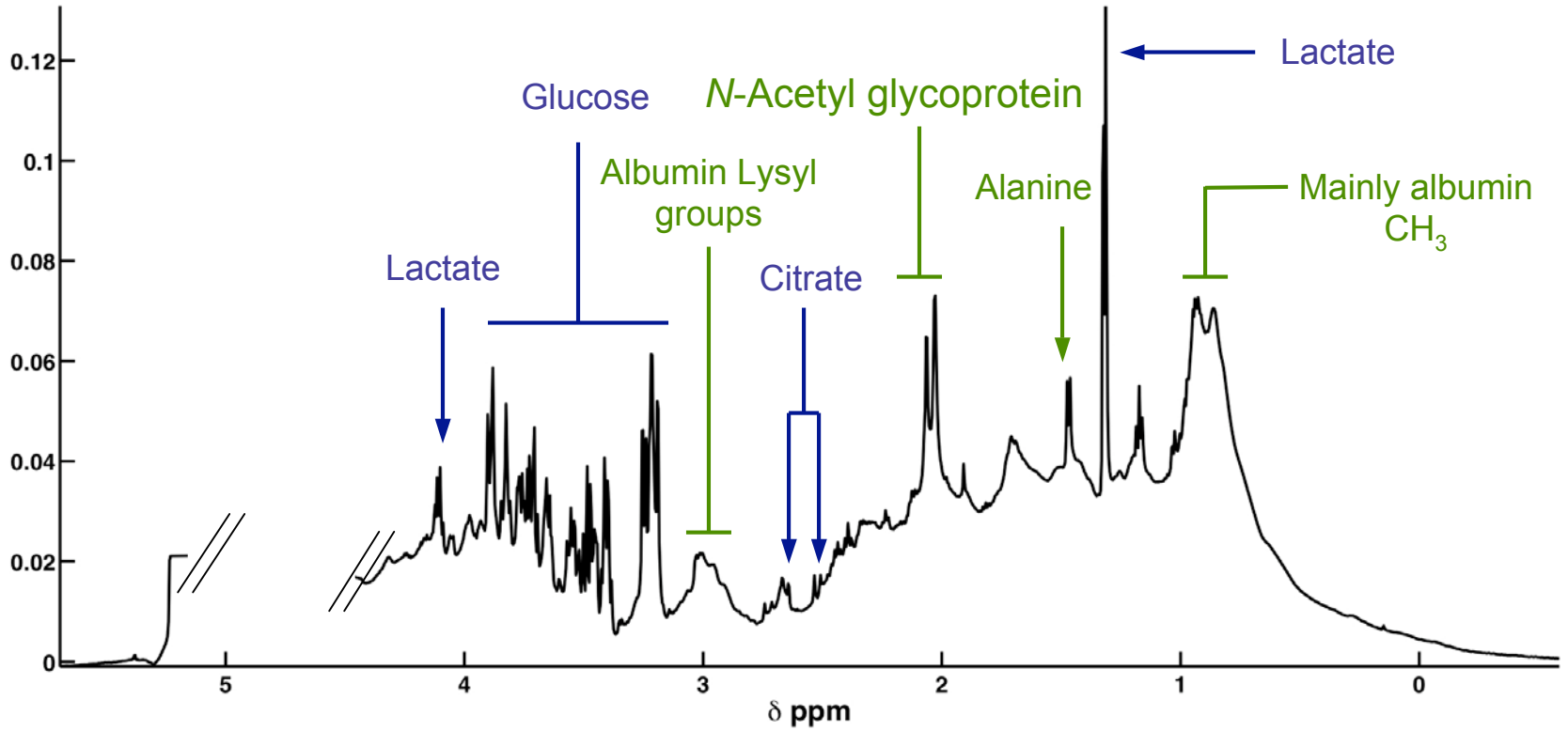
Dominantly Lipidic



Sugar-protein



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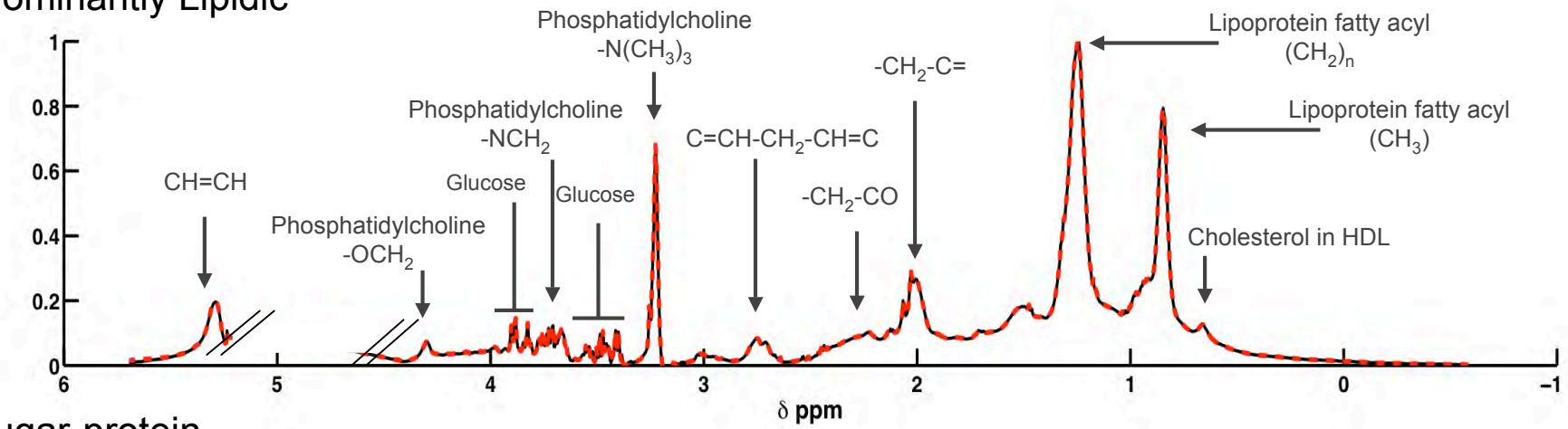
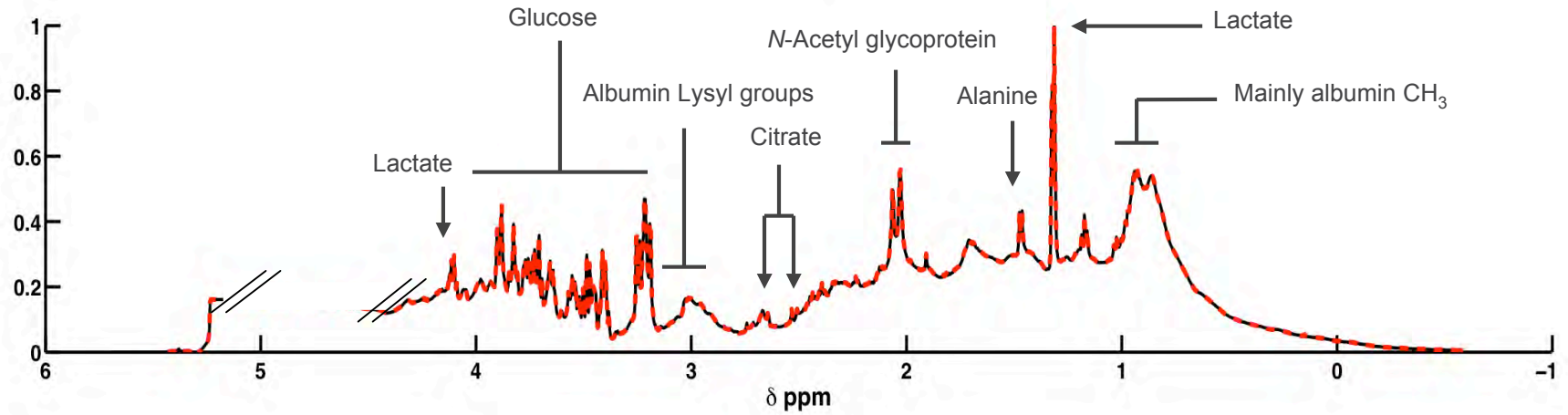
Final SMCR Solution

Dominantly Lipidic



Sugar-protein



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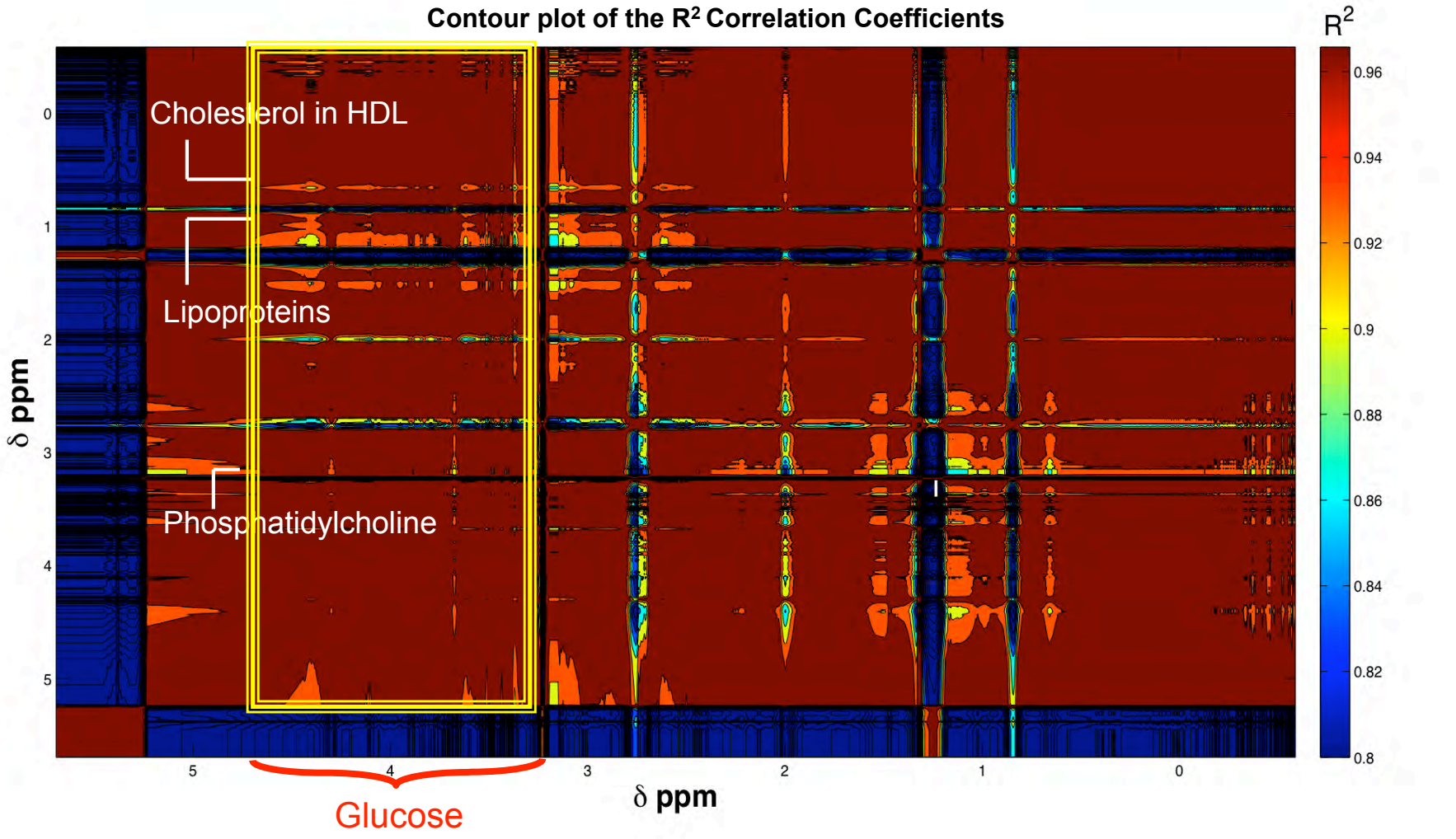
Final SMCR Results



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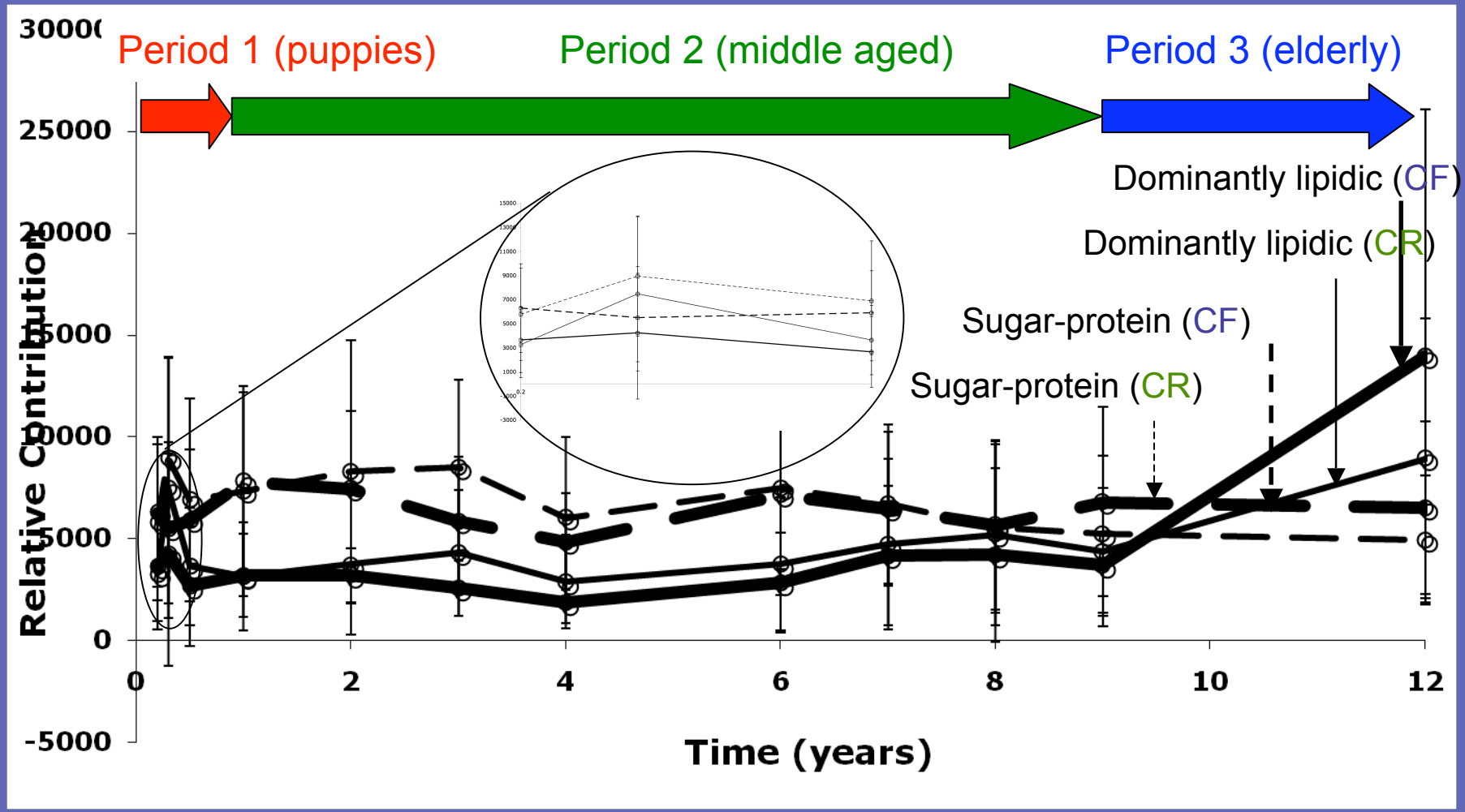
Semi-quantitative trajectory



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Conclusion

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Conclusion

- Identified predominant sources of variation
  - No *a priori information*
- Pinpointed age groups where aging and diet became significant
  - Age groups which were phenotypically different
- Addition of new chemometric tool
  - Metabonomics toolbox
- Diverse application with other biomedical problems
  - Subtle time dependent changes

Richards, S. E.; Wang, Y.; Lawler, D.; Kochhar, S.; Holmes, E.; Lindon, J. C.; Nicholson, J. K. *Analytical Chemistry* **2008**, 80, 4876-4885

Acknowledgments

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