Electronic Supplementary Material (ESI) for Integrative Biology.

Supplementary Figures S1-S10

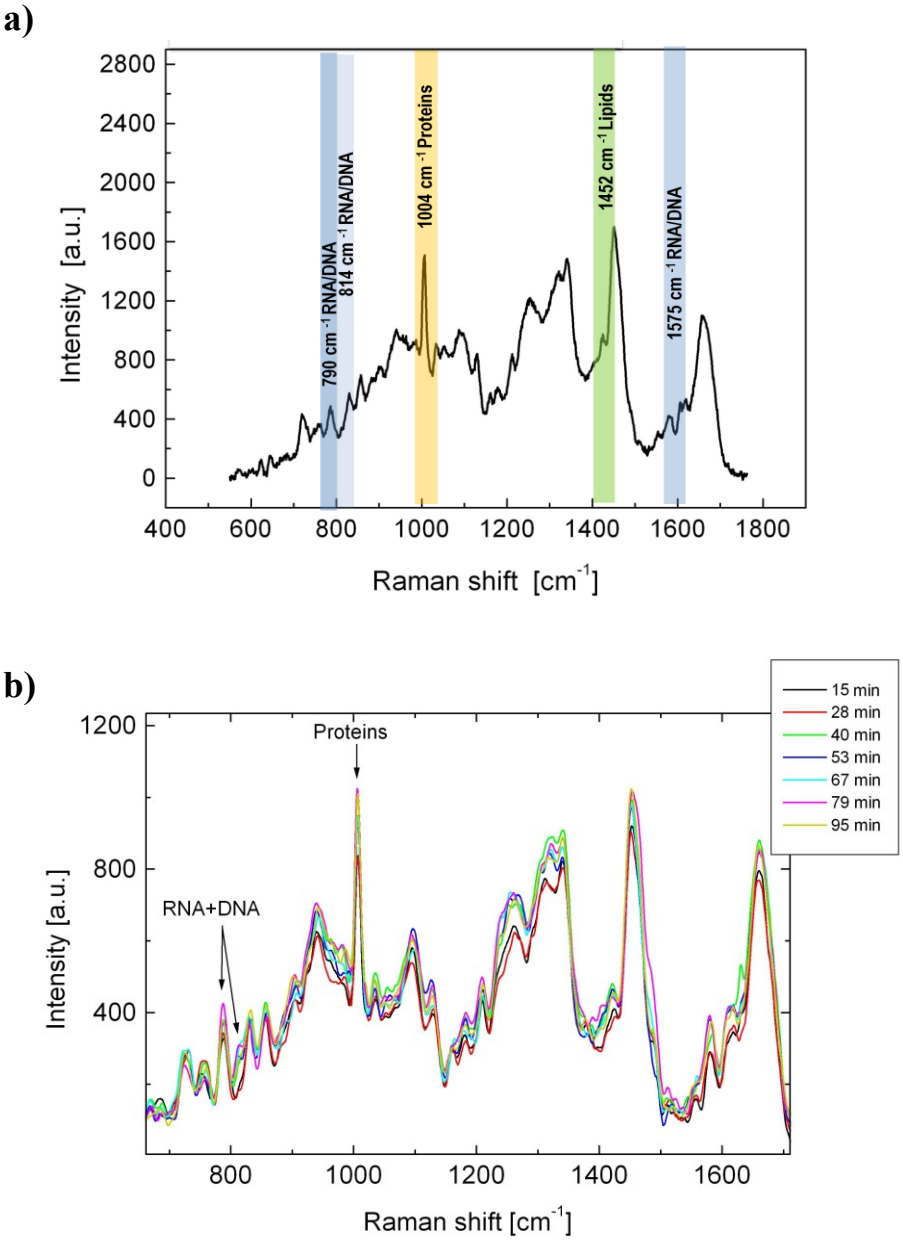


Figure S1: Raman spectra bands assignment. (a) Raman spectrum of the cell nucleus. Light scattering at different molecular groups generates unique spectral bands corresponding to RNA, DNA, proteins and lipids. In particular Raman bands at 790 cm^{-1} and ~814 cm^{-1} and 1575 cm^{-1} are assigned to phosphate diester stretch of RNA and DNA, band at 1004 cm^{-1} is assigned to phenylalanine amino acid and used for concentration measurements of proteins. Band at 1452 cm^{-1} corresponds to C-H bond used for lipids detection. Biomolecular component analysis of the Raman spectra conveys local concentrations of major biomolecular groups such as RNA, DNA, proteins and lipids at each time point (see ref [17a, 33b]). (**b**) Time-lapse series of the Raman Spectra acquired in the nucleoli of live cell in early G1-phase of the cell cycle. Arrows point to Raman spectra bands assigned to nucleic acids and proteins.

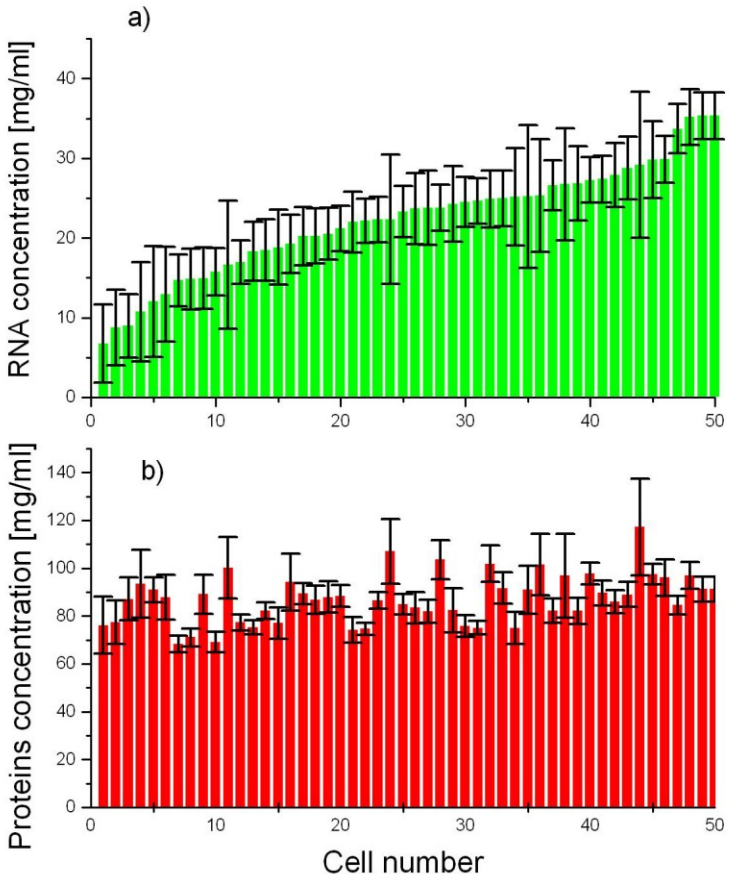


Figure S2: Nucleolar RNA (a) and Proteins (b) concentrations of all measured cells. Data were averaged for each cell throughout the time lapse measurements interval. Error bars represent standard deviations throughout the measurement time. For clarity cells were sorted in the accessing order of RNA concentration.

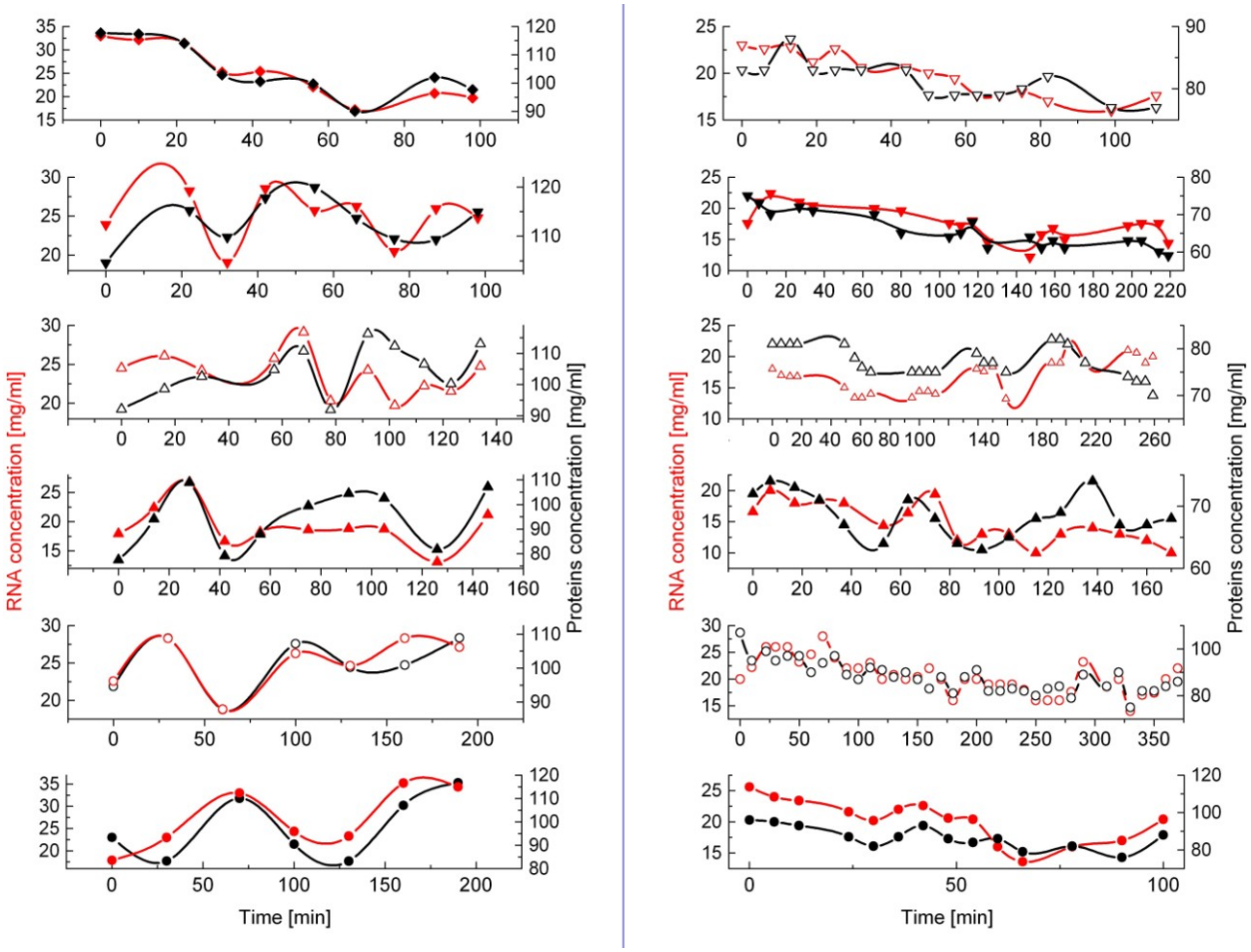


Figure S3: Fluctuations of RNA and protein concentrations in nucleoli of HeLa cells.
Figure illustrates representative data obtained in single cell assays. Raman spectra were acquired in nucleoli of different HeLa cells with different time intervals. Biomolecular component analysis was used to determine concentrations of RNA and proteins at each time point. For each cell RNA concentrations are displayed by red chart and protein concentrations are displayed by black chart. Each chart has two Y-axes for plotting RNA and protein concentrations (mg/ml). RNA concentration values for are plotted at left Y-axis and protein concentrations are plotted at right Y-axis, as indicated.

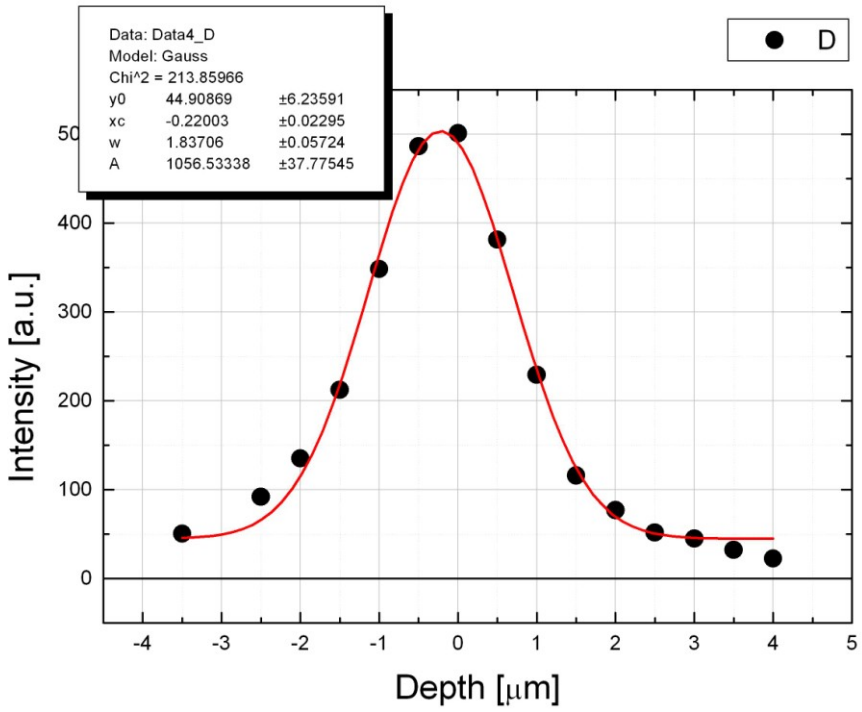


Figure S4: Characterization of the confocal parameter of Raman setup with pinhole of 100μm. Raman signal intensity at 1004 cm^{-1} of polystyrene ~200 nm thin film on glass cover slip versus Z-position of laser beam waist. Confocal parameter is estimated at ~1.8 μm.

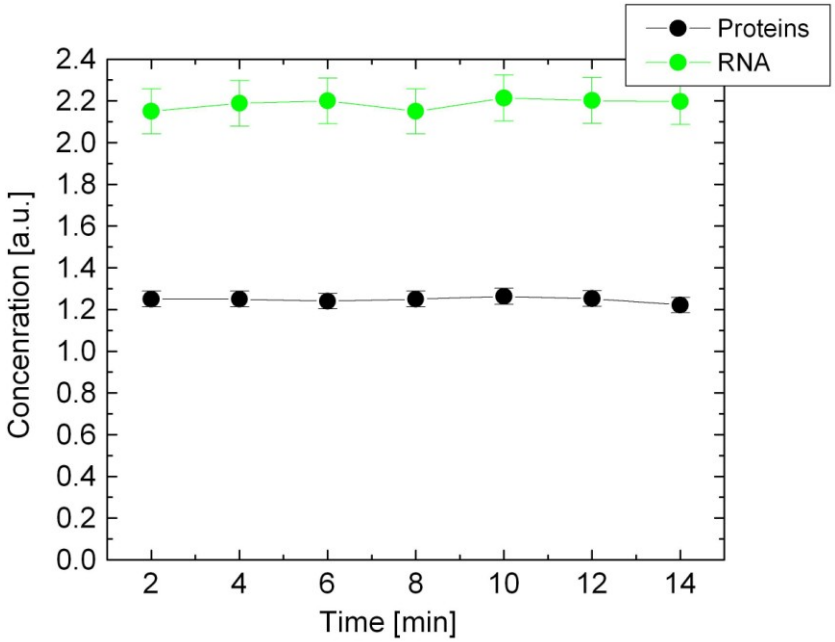
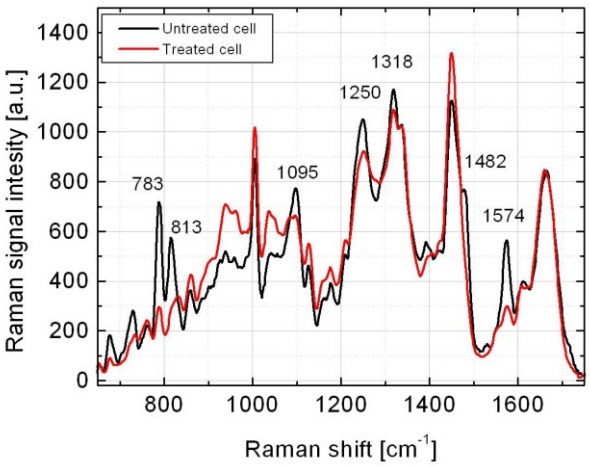
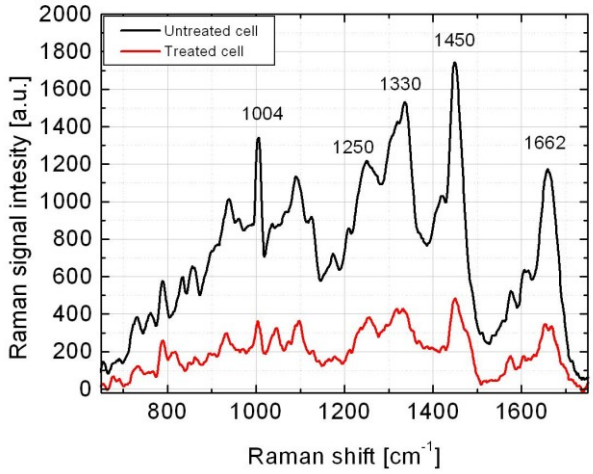


Figure S5: Monitoring of RNA and proteins concentrations in the same nucleolus of fixed HeLa cell. At different time points measured concentrations were almost identical with experimental error not exceeding 3%.

a)



b)



C) Results of Biomolecular Component Analysis (BCA)

Cells	Exp. Conditions	Proteins, mg/ml	DNA, mg/ml	RNA, mg/ml	Lipids, mg/ml
#1	Untreated (Control)	93	0	49	15
	Treated by RNase	94	0	19	25
#2	Untreated (Control)	111	12	14	15
	Proteinase K	30	4	13	5

Figure S6: Changes in intracellular RNA and protein concentrations in response to RNAse and proteinase K treatment. (**a**) Raman spectrum of the nucleolus of a non-treated cell (black) and that of the same nucleolus after 10 min of RNase treatment. Major Raman bands of RNA are indicated by peak frequencies in cm^{-1}. (**b**) Raman spectrum of a randomly selected site in the cell nucleus of non-treated cell (black) and that, acquired in the same nucleus after 10 min of proteinase K treatment. Major Raman bands of proteins are indicated by peak frequencies in cm^{-1}. (**c**) The BCA results, shown in Table 1 demonstrate the expected effect of RNase and Proteinase K treatment on the concentrations of RNA and proteins in the nucleolus.

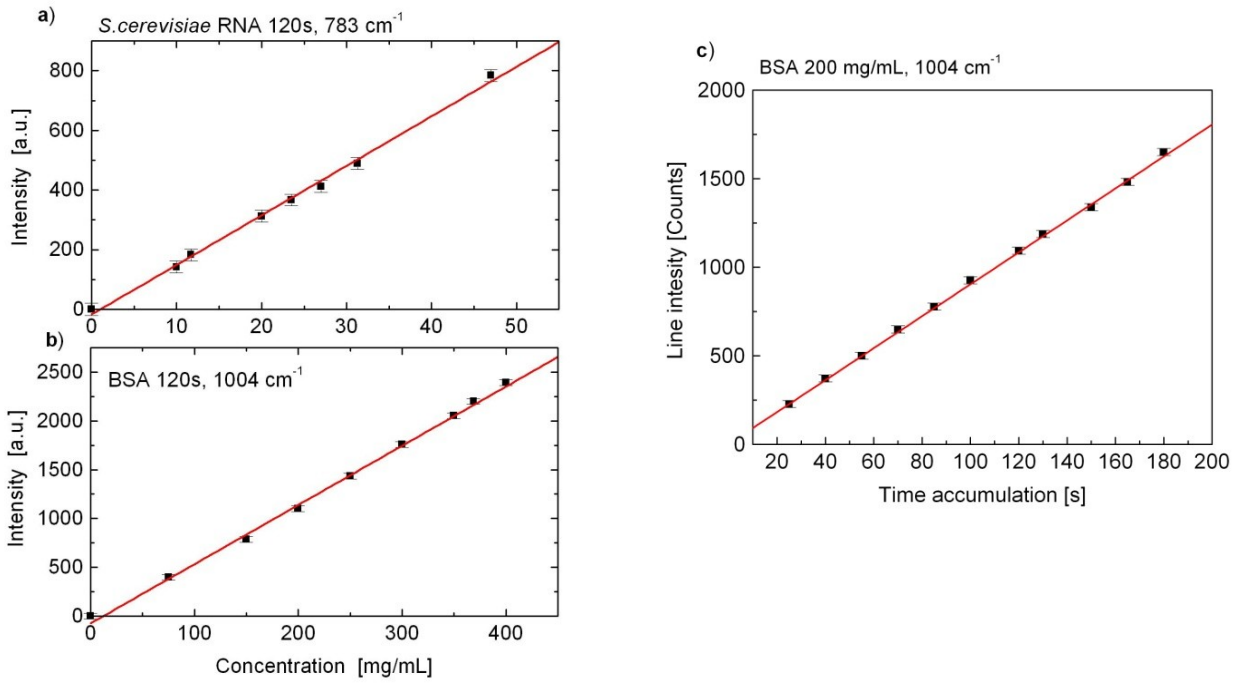


Figure S7: Relation between the Raman signal intensity, concentrations of biomolecules in the studied sample and spectra accumulation time. (a, b) Calibration of Raman signal intensity at different concentrations of RNA and BSA as designated. The chart on (c) demonstrates a linear correlation between the spectra accumulation time and the recorded Raman signal intensity.

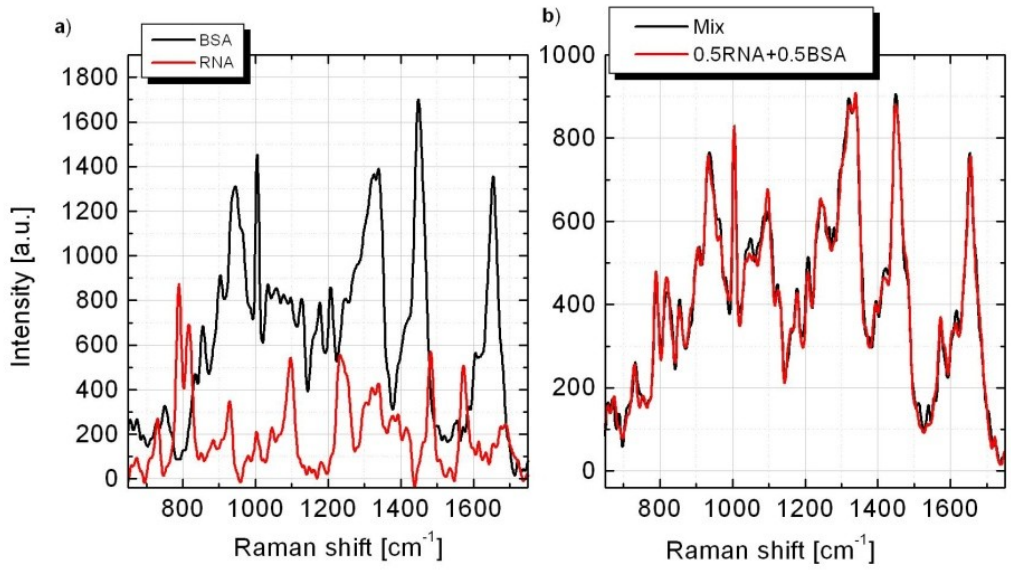


Figure S8: Selective detection and concentration measurements of RNA and proteins in solution by Biomolecular Component Analysis (BCA) (a) Measured Raman spectra of solutions of bovine serum albumin (BSA) (194 mg/ml, black) and tRNA (60 mg/ml, red). (b) Measured Raman spectrum of solution containing 97mg/ml of BSA and 30 mg/ml of tRNA (red). The black curve shows the result of summation of BSA and tRNA spectra in (a) divided by two to model 1:1 mixture spectrum.

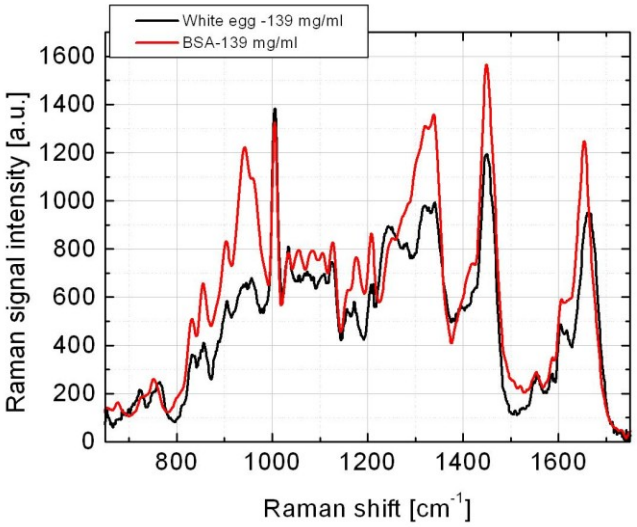


Figure S9: Verification of Bovine serum albumine as a model of proteins in Biomolecular Component Analysis. Chart shows an overlay of Raman spectra of bovine serum albumn (BSA) (red) and white egg (black) sollutions of the same macromolecular concentration. Both, BSA and white egg (composed predominantly of various proteins) demonstrate similar intensity of the Phenylalanine peak at 1004 cm^{-1}, which was used for Biomolecular Component Analysis and Raman setup calibration.

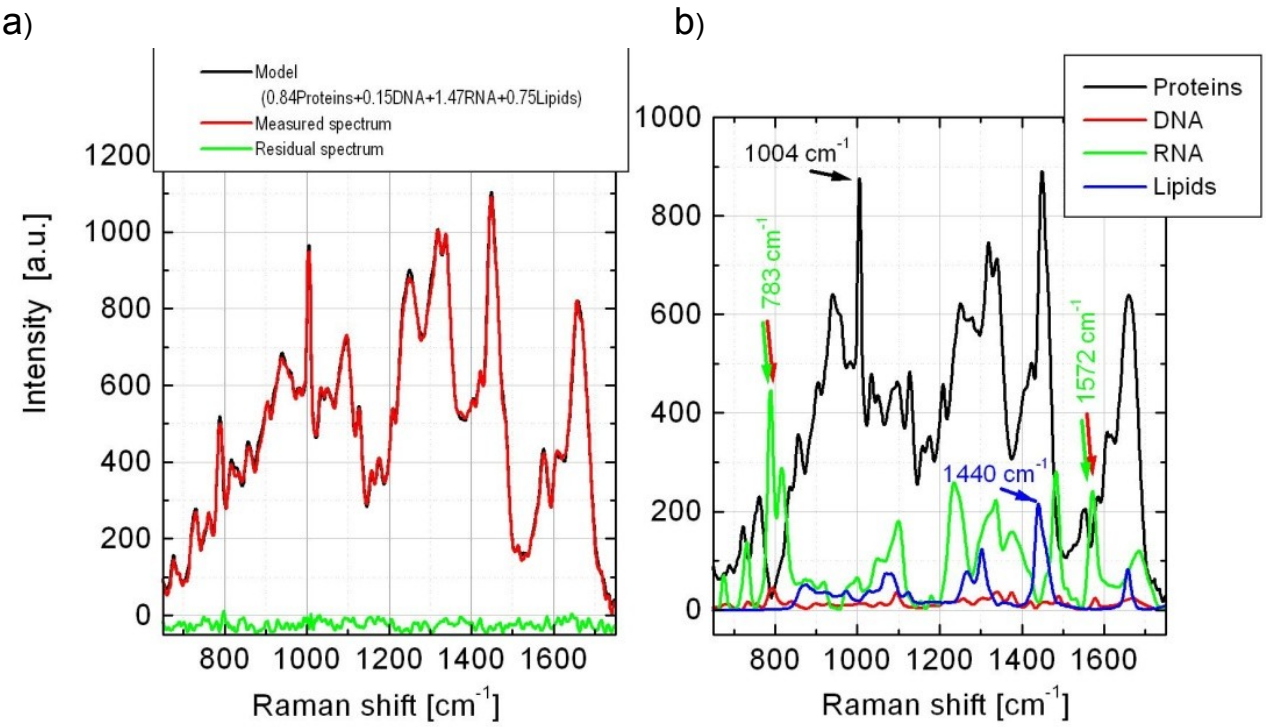


Figure S10: Application of Biomolecular Component Analysis (BCA) for quantitative measurements of Protein, RNA, DNA and lipid concentrations. The BCA algorithm generates in a set of weights for model components (i.e. spectral contribution of proteins, RNA, DNA, lipids) of the measured Raman spectrum. (a) Chart represents the measured spectrum (red); the Model spectrum, obtained through summation of weight component spectra; and the Residual spectrum (green) obtained by subtraction of the Model spectrum from the measured spectrum. The residual spectrum is comparable to noise, which confirms the accuracy of BCA modeling.

(b) BCA-generated model components of the measured Raman spectrum from (a) are shown. Arrows point at characteristic frequencies used for measurements of concentrations of proteins, RNA, DNA and lipids. Based on concentration calibration of Raman setup the concentrations of each type of biomolecule are calculated. For details see ref [17a, 33b].