



Raman Spectroscopic Characterization of Anomalous Intravascular Fibrous Casts: Evidence for Stage-Dependent β -Sheet Enriched Protein Maturation

Daniel Santiago, RPh, PharmD ¹, Greg Harrison ², Miklós Veres ³, Mark File ⁴, Dennis Planner ⁵

¹Independent Researcher, Orlando, FL USA.

²Chemist, Independent researcher, Australia.

³Senior Scientist, Hun-REN Wigner Research Centre for Physics, Hungary.

⁴Chemist, PhD Candidate, Hungary.

⁵Chemical Engineer, Hungary.

*Corresponding Author: Daniel Santiago, RPh, PharmD

Received: 06 June 2026;

Accepted: 24 June 2026;

Published: 01 July 2026

Abstract

White-to-pale, rubbery intravascular fibrous casts recovered during routine embalming were analyzed using Raman micro-spectroscopy (633 nm and 785 nm excitation), the Kjeldahl method for total crude protein estimation, and ion-exchange chromatography for amino-acid profiling. Raman spectra from both specimens exhibited strong protein signatures, with notable differences in the Amide I/Amide III intensity ratio and the presence of a 1620 cm^{-1} Amide I sub-band. Expert Raman analysis distinguishes a native-like, predominantly α -helical configuration in one specimen from a more β -sheet-enriched, aggregation-advanced state in the other (S1 vs S2). Additional spectral markers in the β -sheet-enriched specimen, including sharper aromatic ring-breathing modes and phosphorylation-associated bands, suggest a more ordered and stabilized secondary-structure arrangement. Kjeldahl analysis measured total crude protein at 7.72 m/m% for the analyzed sample, and ion-exchange amino-acid profiling returned the composition listed in Results. The combined spectroscopic and compositional data indicate that these casts represent atypical protein aggregates exhibiting heterogeneity consistent with stage-dependent β -sheet enrichment, distinct from conventional postmortem thrombi. Although donor histories were unavailable, the distinct physical and spectroscopic features of these casts may aid in their recognition during embalming and postmortem examination.

Keywords: *Raman micro-spectroscopy; intravascular casts; β -sheet enrichment; protein aggregates; Kjeldahl analysis; ion-exchange chromatography; secondary structure; embalming; forensic pathology.*

Introduction

Since 2021, embalmers have reported anomalous intravascular fibrous casts encountered during routine preparation of the deceased and a multi-year international survey documents such self-reported observations across five countries from 2022–2025 [1]. These white-to-pale, rubbery, elongated structures differ markedly from conventional postmortem thrombi in morphology and mechanical behavior, exhibiting unusual tensile strength, elasticity, and resistance to routine lysis procedures. Their biochemical composition and structural organization remain incompletely characterized, and systematic analytical data are limited [2,3].

Raman micro-spectroscopy provides a non-destructive means of probing the molecular architecture of proteinaceous materials. Its sensitivity to amide vibrational modes enables discrimination among α -helical, disordered, and β -sheet-enriched secondary-structure states, and it has been widely applied to the study of fibrillar and aggregated protein assemblies [4-6]. Complementary Kjeldahl determination of total crude protein and ion-exchange chromatography for amino-acid profiling offer orthogonal measures of bulk protein composition, permitting assessment of amino-acid enrichment patterns that may correlate with atypical structural organization.

In this study, we characterize representative specimens of the fibrous intravascular material using Raman micro-spectroscopy alongside Kjeldahl protein quantitation and

amino-acid analysis. Our objective was to determine whether integrated structural and compositional signatures align with conventional postmortem clot biology or instead support classification of the material as atypical, β -sheet-enriched protein aggregates. The data presented here indicate that these signatures are inconsistent with conventional postmortem thrombi and instead support classification of the casts as atypical protein aggregates with non-canonical secondary-structure organization exhibiting heterogeneity consistent with stage-dependent β -sheet enrichment.

Materials and Methods

Sample origin and handling

The anomalous intravascular fibrous material examined in this study was recovered incidentally during routine embalming procedures performed in standard mortuary practice. These structures were removed as part of the normal preparation required to clear vascular obstructions to permit effective administration of embalming fluid. The material was obtained postmortem under standard donor or next-of-kin authorization procedures and in accordance with local mortuary regulations. All samples were anonymized and de-identified prior to analysis and contained no identifiable personal information. Because this work involves only de-identified cadaveric material collected during routine embalming and no interaction with living subjects, it was considered exempt from Institutional Review Board oversight under applicable regulations (e.g., 45 CFR 46).

Raman micro-spectroscopy

Raman measurements were performed at the HUN-REN Wigner Research Centre for Physics (Budapest, Hungary) on two solid specimens (S1 and S2) delivered in sealed vials. Particles a few millimeters in size were removed from residual liquid, placed onto

a silicon-wafer substrate, and allowed to dry completely at ambient conditions. Spectra were acquired using a Renishaw InVia micro-Raman spectrometer with 633 nm and 785 nm excitation lasers and a 50 $\times$ objective (NA = 0.9), yielding an excitation spot diameter of approximately 2–4 μ m. Spectra were collected over 200–3100 cm^{-1} (633 nm) and 200–3200 cm^{-1} (785 nm). A polynomial baseline subtraction was applied to remove fluorescence background. For each specimen and excitation wavelength, five spectra were recorded at distinct locations to assess intra-sample variability. Representative measurement positions and raw spectra are shown in the figures; the full spectral datasets and expert interpretation are provided in the Supplementary Material. Raman criteria and peak assignments follow established literature for protein secondary-structure analysis and fibrillar aggregates [4,5].

Protein and amino-acid analysis

Total crude protein content was determined by the Kjeldahl method at the University of Debrecen (Debrecen, Hungary) following MSZ EN ISO 5983-2:2009 (report K24/153; Supplementary Material). Amino-acid composition was determined by ion-exchange chromatography following acid hydrolysis (MSZ EN ISO 13903:2005). Results are reported as mass-per-mass percentages (m/m%) for each amino-acid species. Comparative literature values for fibrin(ogen) and postmortem clot composition were consulted [7-9].

Macroscopic appearance

The dried specimens differed visibly in color: S1 appeared light brown–yellow, whereas S2 was dark brown (**Figure 1**). Measurement spots selected for Raman analysis showed corresponding textural heterogeneity, with S1 exhibiting smoother, lighter regions and S2 displaying darker, more granular surfaces (**Figure 2**).

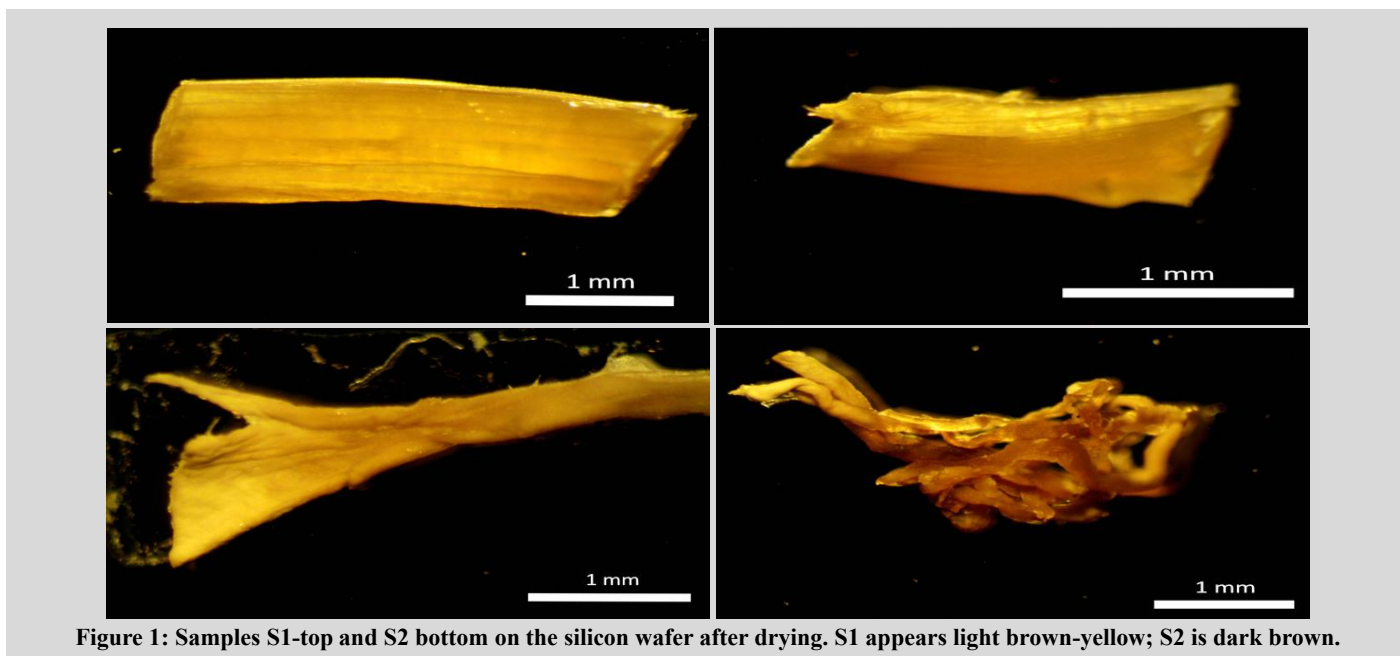


Figure 1: Samples S1-top and S2 bottom on the silicon wafer after drying. S1 appears light brown-yellow; S2 is dark brown.

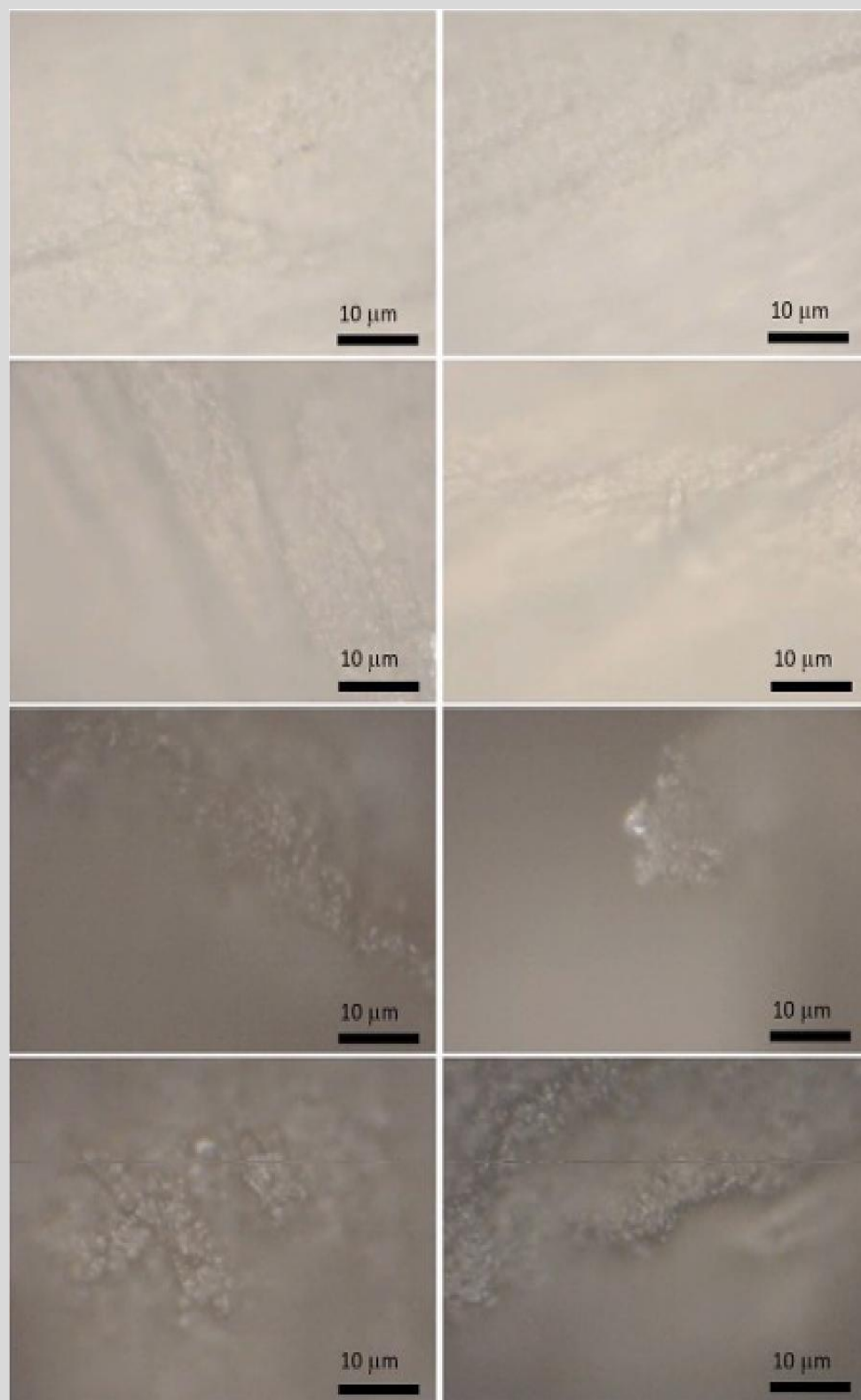


Figure 2: Typical morphology of the S1 and S2 samples around the measurement spots.

Results

Raman spectroscopy

Raman spectroscopy at 633 nm

Spectra acquired at 633 nm exhibited clear protein signatures in both specimens (**Figure 3**). Prominent C–H stretching bands at ~ 2870 , 2930 , and 2970 cm^{-1} indicated contributions from aliphatic and aromatic side chains. The fingerprint region was dominated by

Amide I ($\sim 1655\text{ cm}^{-1}$) and Amide III ($\sim 1255\text{ cm}^{-1}$) vibrations, consistent with peptide backbone structure. Additional marker bands included phenylalanine ($\sim 1000\text{ cm}^{-1}$) and tryptophan ($\sim 752\text{--}753\text{ cm}^{-1}$). Across the series, S2 displayed more intense and more numerous Raman features than S1, consistent with the expert report's observation of greater spectral definition in the darker specimen. These Raman-based distinctions reflect differences in secondary-structure organization and spectral order but do not by themselves establish the presence of canonical amyloid fibrils [4].

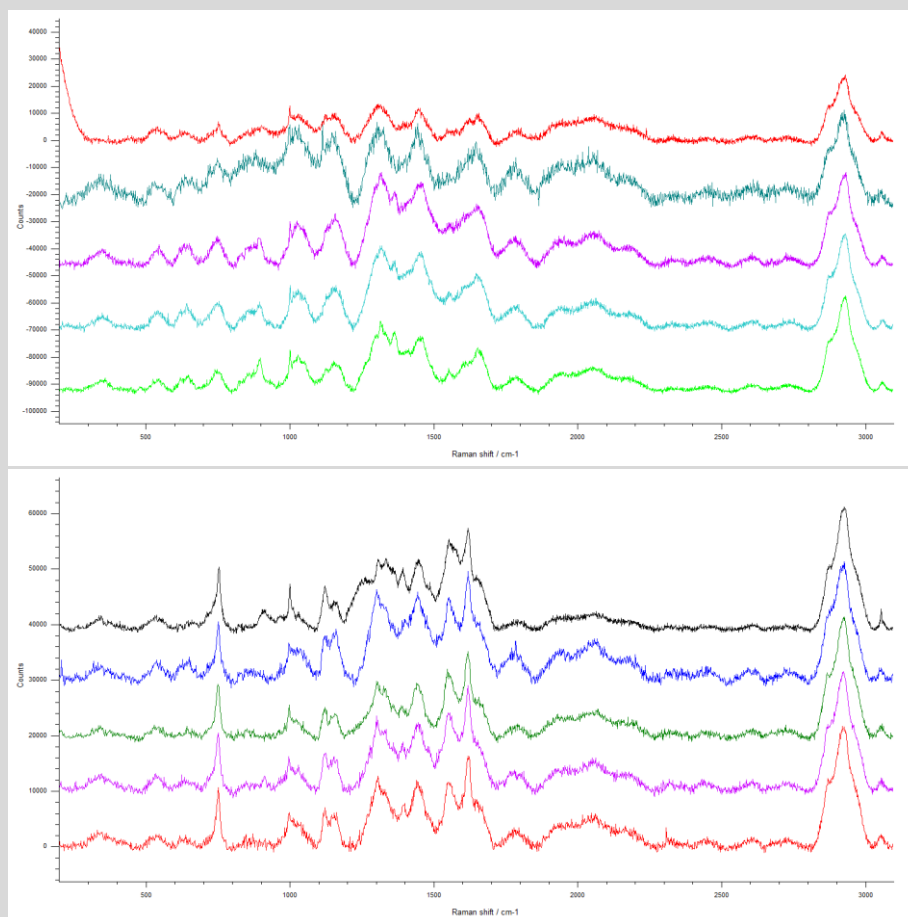


Figure 3: Series of Raman spectra recorded on the S1 (top) and S2 (bottom) samples with 633 nm excitation.

Representative spectra (**Figure 4**) revealed systematic differences between S1 and S2. The most diagnostic feature was the Amide I/Amide III intensity ratio, which was markedly higher in S1 and lower in S2. Additional differentiating peaks were observed at $\sim 750\text{ cm}^{-1}$, 896 cm^{-1} , 1122 cm^{-1} , 1365 cm^{-1} , 1395 cm^{-1} , and 1621 cm^{-1} . These variations are consistent with differences in local

chemical environment and backbone organization between the two specimens. Detailed peak assignments, spectral fitting parameters, and full raw datasets are provided in the Supplementary Raman report; Raman criteria and assignments follow established literature for protein secondary-structure analysis and fibrillar aggregates [5,6].

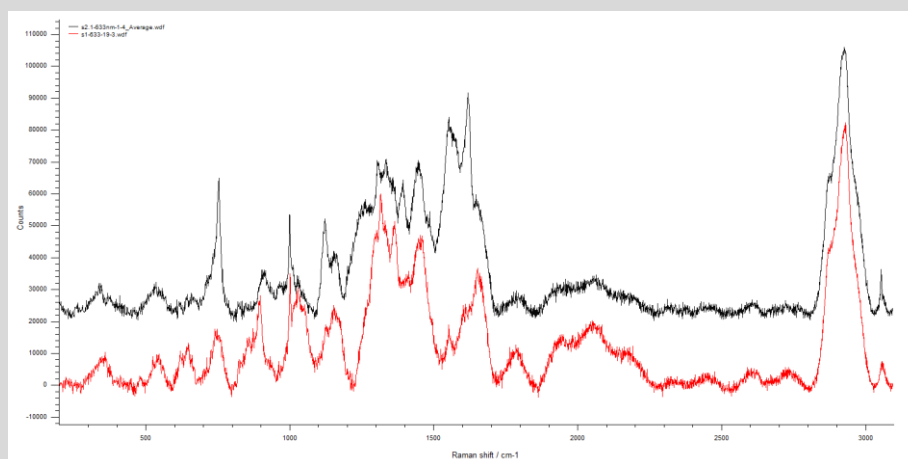


Figure 4: Comparison of typical 633 nm excited Raman spectra of the S1 (red) and S2 (black) samples.

Raman spectroscopy at 785 nm

Spectra collected at 785 nm (**Figure 5**) showed broadly similar protein features in both specimens, including Amide I, Amide III, and C–H stretching bands. Differences between S1 and S2 were present but less pronounced than at 633 nm (**Figure 6**), consistent

with reduced resonant enhancement of specific structural motifs at the longer excitation wavelength [4]. Representative spectra and comparative plots are provided in the Supplementary Material; peak assignments and fitting parameters follow established criteria for protein secondary-structure analysis.

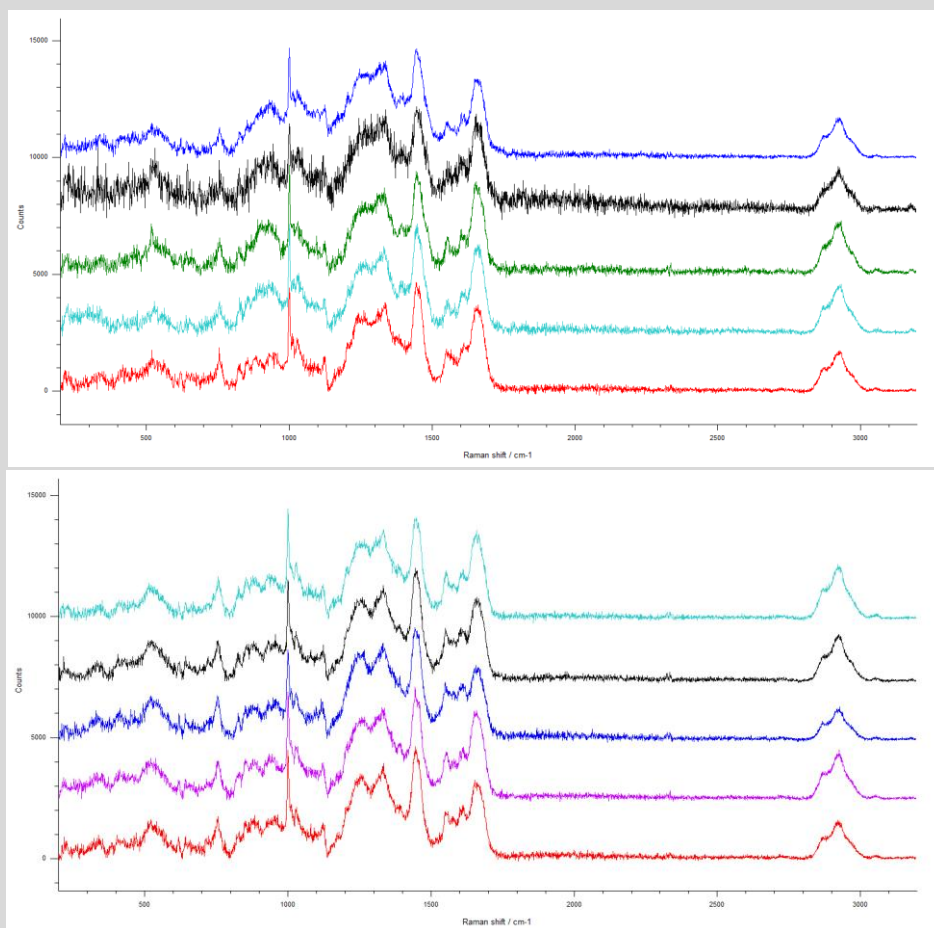


Figure 5: Series of Raman spectra recorded on the S1 (top) and S2 (bottom) samples recorded with 785 nm excitation.

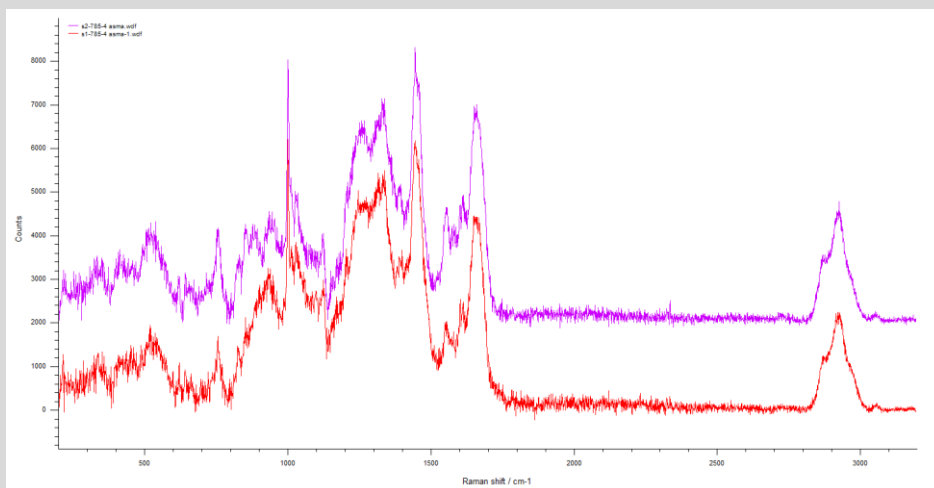


Figure 6: Comparison of typical 785 nm excited Raman spectra of the S1 (red) and S2 (purple) samples.

Raman Spectral Interpretation

Interpretation of structural differences

Independent expert analysis interpreted the Amide I/Amide III intensity ratio as indicative of secondary-structure organization. The higher ratio observed in S1 is consistent with a native-like, predominantly α -helical configuration (pre-fibrillar or early-intermediate state), whereas the lower ratio in S2, together with the pronounced Amide I sub-band at $\sim 1620\text{ cm}^{-1}$, corresponds to a more advanced, β -sheet-enriched configuration. These interpretations follow established Raman criteria for differentiating ordered versus disordered protein aggregates; detailed peak assignments and spectral fitting parameters are provided in the Supplementary Raman report.

Amino-acid composition (Kjeldahl analysis)

Kjeldahl and ion-exchange analysis of a representative sample (Table 1) yielded the following notable values: proline 1.55 m/m%, lysine 1.18 m/m%, and cysteine 0.01 m/m%. The overall amino-acid profile differs from typical literature values reported for conventional postmortem fibrin clots and many characterized amyloid proteins [7,10,11]. Elevated proline may favor β -turns or irregular motifs, while the extremely low cysteine level is consistent with limited disulfide-bonded stabilization. These compositional features are concordant with the Raman-derived evidence for non-canonical secondary-structure organization. The spectroscopist also noted broad, inconclusive features in the 480–570 cm^{-1} and 680–790 cm^{-1} regions that could reflect weak disulfide or C–S contributions, but these bands were not definitive.

Analytical caveat

The reported amino-acid percentages represent the hydrolyzable protein fraction of the fibrous casts and do not quantify non-proteinaceous components or the entire sample mass; absolute percentages are therefore low by design.

Integrated interpretation

Taken together, the Raman-derived structural signatures and the Kjeldahl-derived compositional profile indicate that the examined material represents an atypical, protein-containing aggregate with non-canonical secondary-structure organization [4,5,12]. The data are consistent with staged-dependent β -sheet enrichment, progressing from a native-like, α -helical state in S1 toward a more β -sheet-enriched, aggregation-advanced state in S2. While these observations support classification of the casts as atypical protein aggregates distinct from conventional postmortem thrombi, they do not by themselves establish the presence of canonical amyloid fibrils; complementary ultrastructural or immunochemical assays would be required to confirm amyloid morphology or specific protein identity [10,13].

Table 1: Amino Acid composition (Kjeldahl method) *

Amino Acid	Content (m/m%)
Aspartic acid (Asp)	1.19
Threonine (Thr)	0.27
Serine (Ser)	0.58
Glutamic acid (Glu)	0.60
Proline (Pro)	1.55
Glycine (Gly)	0.37
Alanine (Ala)	0.21
Cysteine (Cys)	0.01
Valine (Val)	0.20
Methionine (Met)	0.05
Isoleucine (Ile)	0.16
Leucine (Leu)	0.33
Tyrosine (Tyr)	0.05
Phenylalanine (Phe)	0.07
Histidine (His)	0.22
Lysine (Lys)	1.18
Arginine (Arg)	0.26

*Notes (translated from laboratory report originally written in Hungarian): 1. The sum of the tested amino-acids does not equal 100%. the reported sum represents only the total of the individually measured amino-acids. 2. The amino acid profile reflects the composition of the protein fraction (or the measurable portion thereof) and does not represent the composition of the entire sample.

Discussion

Raman evidence for differential secondary-structure organization

Raman micro-spectroscopy of the two anomalous intravascular fibrous specimens produced protein-dominant spectra characterized by prominent Amide I and Amide III vibrations. The principal spectral distinction was the Amide I/Amide III intensity ratio: a relatively high ratio in S1 is consistent with a native-like, predominantly α -helical configuration (pre-fibrillar or early-intermediate state), whereas the lower ratio in S2, together with a pronounced Amide I sub-band at $\sim 1620\text{ cm}^{-1}$, indicates a shift toward intermolecular β -sheet enrichment [4,5]. Detailed peak assignments and spectral fitting parameters are provided in the Supplementary Raman report. These spectroscopic differences

plausibly underlie the variable mechanical resilience and persistence reported for these casts during embalming.

Additional spectral markers

S2 exhibited sharper aromatic ring-breathing modes and clearer features in the $1360\text{--}1450\text{ cm}^{-1}$ region, consistent with increased spectral order and backbone stabilization. Broad, low-intensity bands in the $480\text{--}570\text{ cm}^{-1}$ and $680\text{--}790\text{ cm}^{-1}$ regions may reflect weak disulfide or C–S contributions, but their breadth and overlap preclude definitive assignment by Raman alone. Weak signals in the $900\text{--}1100\text{ cm}^{-1}$ region could indicate phosphate-related vibrations; however, overlap with the strong phenylalanine band near 1000 cm^{-1} limits interpretive certainty [9,14]. These observations underscore the chemical complexity of the material and the need for orthogonal chemical and structural assays.

Amino-acid composition and structural implications

Kjeldahl and ion-exchange analysis of a representative specimen revealed elevated proline (1.55 m/m%) and lysine (1.18 m/m%) and markedly low cysteine (0.01 m/m%). Elevated proline can favor β -turns or irregular motifs that facilitate aggregation, while minimal cysteine suggests limited disulfide-bonded stabilization. The amino-acid profile departs from published compositions of human fibrinogen/fibrin and many characterized amyloid proteins, supporting the interpretation of a non-canonical protein aggregate [7,10]. These compositional data are concordant with the Raman-derived evidence for altered secondary-structure organization but do not identify the constituent protein(s).

Integrated interpretation and limits of spectroscopic terminology

Taken together, the Raman and amino-acid data support classification of the examined material as atypical, protein-containing aggregates exhibiting a spectroscopic maturation pattern from a native-like, α -helical state (S1) toward a more β -sheet-enriched, aggregation-advanced state (S2) [4,5]. This description denotes a spectroscopic phenotype rather than a definitive ultrastructural or molecular identification. β -Sheet enrichment alone is not diagnostic of canonical amyloid; confirmation of cross- β fibrillar architecture requires orthogonal methods such as transmission electron microscopy, X-ray fiber diffraction, or Congo red birefringence with appropriate controls [10,13].

Context, alternative explanations, and prior observations

Stage-dependent aggregation from soluble or native-like states to β -sheet-rich assemblies is well documented for many proteins and can alter mechanical properties and proteolytic resistance, offering a plausible framework for the persistence of these casts [15]. Exogenous or endogenous proteins can persist in tissues under particular circumstances, but no protein-specific identification or immunohistochemistry was performed on the casts in this study, and donor exposure or infection histories are unknown [16]. Consequently, no causal link to any exposure, infectious agent, vaccine, or therapeutic intervention can be inferred from the present data.

Table 2: Main Raman bands observed in the S1 and S2 samples and their vibrational assignments.

Raman peak position (cm ⁻¹) Sample		Assignment
S1	S2	
3060	3060	sp ² CH stretching (aromatic)
2970	2970	Antisymmetric sp ³ CH ₃ stretching

2930	2930	Antisymmetric sp^3 CH_2 stretching
2870	2870	Symmetric sp^3 CH_3 stretching
1655	1648	Amide I ($\text{C}=\text{O}$ stretch)
-	1620	Amide I, intermolecular β -sheet ($\text{C}=\text{O}$ stretch)
1550	1553	Amide II ($\text{N}-\text{H}$ bend + $\text{C}-\text{N}$ stretch)
1460	1450	sp^3 CH_2 and sp^3 CH_3 deformation
-	1393	COO^- symmetric stretch / CH_3 deformation
1363	-	Tryptophan W7
-	1335	CH deformation
1315	1315	CH_2 twist
1256	1255	Amide III ($\text{N}-\text{H}$ bend + $\text{C}-\text{N}$ stretch)
-	1121	$\text{C}-\text{N}$ / $\text{C}-\text{O}-\text{P}$ stretch (phosphoester)
1030	1030	Phenylalanine deformation
1000	1000	Aromatic ring-breathing, phenylalanine
893	-	$\text{C}-\text{C}$ stretch
744	753	Tryptophan W18
647	647	Tyrosine deformation
620	620	Phenylalanine deformation
545	545	$\text{S}-\text{S}$ stretch (disulfide)

Notes: Dashes (—) indicate the band is absent or not prominent in that sample. Assignments follow the detailed expert analysis in the supplementary Raman report

Implications for mortuary and forensic practice

The physical and spectroscopic properties described here may explain difficulties in clearing vascular obstructions and achieving uniform embalming fluid distribution. Recognition of these casts as proteinaceous aggregates with variable structural maturity could inform mortuary technique, targeted vessel manipulation, alternative injection strategies, or extended flushing protocols, and aid pathologists in distinguishing atypical intravascular casts from conventional postmortem thrombi [17].

Limitations and directions for future research

This study is descriptive and limited by small sample size, incomplete provenance, and lack of matched controls. Postmortem changes, embalming chemicals, preexisting pathology, or idiosyncratic biological factors may have influenced the observed features. Raman spectroscopy and Kjeldahl amino-acid profiling provide complementary structural and compositional information but cannot determine fibril ultrastructure, definitive protein identity, or formation mechanism. Future work should include larger, well-documented cohorts with matched controls, blinded analyses, and orthogonal methods, proteomics for protein identification, immunohistochemistry for localization, electron microscopy and X-ray fiber diffraction for ultrastructure, and biochemical assays to probe cross-linking and post-translational modifications, while avoiding premature causal attribution.

Conclusion

Raman micro-spectroscopy revealed clear protein signatures with marked differences in secondary-structure organization between specimens, most notably a divergence in the Amide I/Amide III intensity ratio: a high ratio in S1 consistent with a native-like, α -helical state and a low ratio together with a $\sim 1620\text{ cm}^{-1}$ band in S2 indicative of progression toward β -sheet-enriched organization [4,5]. Complementary Kjeldahl amino-acid profiling demonstrated an atypical compositional pattern, elevated proline and lysine with

minimal cysteine, that supports interpretation of a non-canonical, protein-containing aggregate rather than a typical fibrin clot [7,10].

Taken together, these data provide structural evidence of heterogeneity consistent with stage-dependent β -sheet enrichment within these atypical protein aggregates, with S1 representing an earlier or native-like state and S2 showing features of a more advanced β -sheet-enriched assembly. This apparent staged progression offers a plausible explanation for the casts' persistence during embalming. The analyses are descriptive and limited in scope: they do not identify specific protein constituents, determine ultrastructural architecture, or establish formation pathways.

These results underscore the need for systematic, multi-modal investigation. Future studies incorporating proteomics, histochemistry, electron microscopy, controlled comparisons, and well-documented provenance will be essential to clarify biochemical identity, structural evolution, prevalence, and potential clinical or postmortem significance.

Declarations

Ethical Statement

The anomalous intravascular fibrous material examined in this study was recovered incidentally during routine embalming procedures performed on deceased individuals in standard mortuary practice. The material was obtained as part of the normal preparation process required to clear vascular obstructions (such as clots or plugs) for effective administration of embalming fluid.

This study involves only postmortem cadaveric material and does not constitute human subjects research as defined by applicable regulations (e.g., 45 CFR 46). Therefore, it is exempt from Institutional Review Board (IRB) or ethics committee review. The handling and use of this material complies with ethical standards for cadaveric tissues, including respect for the dignity of the deceased. Authorization for embalming procedures and the handling of incidental materials generated during embalming was obtained through standard donor or next-of-kin consent processes in accordance with local regulations and mortuary protocols. All analyses were performed on anonymized samples with no linked personal identifiers.

Author Contributions

Miklós Veres: Conceptualization, Raman spectroscopic analysis and data interpretation.

Daniel Santiago: Conceptualization, Writing – Original Draft, Writing – Review & Editing, Formal analysis.

Greg Harrison: Conceptualization, Sample acquisition, Project administration, Formal analysis.

Dennis Planner and Mark File: Conceptualization, Writing – Review & Editing.

Funding

The author(s) reported there is no funding associated with the work featured in this article.

AI Disclosure

The authors used artificial intelligence, assisted tools solely for reference formatting and citation verification. All original ideas, analyses, interpretations, and textual content were conceived and written by the authors.

Acknowledgments.

The full Raman report (including all raw spectra, expert peak assignments, and commentary on proposed interpretations) is available as supplementary material A.

We also thank the mortician who provided the samples for this study.

Supplementary Material Available

Full Raman spectroscopic report including raw spectra (633 nm & 785 nm), peak-by-peak assignments, expert commentary on proposed forensic interpretations, and high-resolution photographs. This detailed report provides the complete underpinning for Figures 1–6 and the structural interpretations presented herein.

References

- [1] Haviland, T. F., Kasner, L., & Santiago, D. (2026). Self-reported observations of unusual white fibrous structures in embalmed corpses: Multi-year survey results from embalmers in five countries, 2022–2025. Zenodo. <https://doi.org/10.5281/zenodo.20725487>
- [2] Kell, D. B., Laubscher, G. J., & Pretorius, E. (2022). A central role for amyloid fibrin microclots in long COVID/PASC: Origins and therapeutic implications. *Biochemical Journal*, 479(4), 537–559. <https://doi.org/10.1042/BCJ20220016>
- [3] Schofield, J., Abrams, S. T., Jenkins, R., Mozdiak, E., Edwards, J., & Toh, C. H. (2024). Microclots, as defined by amyloid-fibrinogen aggregates, predict risks of disseminated intravascular coagulation and mortality. *Blood Advances*, 8(10), 2499–2508. <https://doi.org/10.1182/bloodadvances.2023012473>
- [4] Blat, A., Dybaś, J., Chrabaszcz, K., Bulat, K., Jasztal, A., Marzec, K. M., & Małek, K. (2019). FTIR, Raman and AFM characterization of the clinically valid biochemical parameters of the thrombi in acute ischemic stroke. *Scientific Reports*, 9, Article 15475. <https://doi.org/10.1038/s41598-019-51932-0>
- [5] Ramos, S., Santos, T., Silva, A., & Coimbra, P. (2024). Raman spectroscopy in the study of amyloid formation and amyloidosis. *International Journal of Molecular Sciences*, 25(15), Article 8214. <https://doi.org/10.3390/ijms25158214>
- [6] Mohd Nor Ihsan, N. S., Abdul Sani, S. F., Looi, L. M., Cheah, P. L., Chiew, S. F., Pathmanathan, D., & Bradley, D. A. (2023). A review: Exploring the metabolic and structural characterisation of beta pleated amyloid fibril in human tissue using Raman spectrometry and SAXS. *Progress in biophysics and molecular biology*, 182, 59–74. <https://doi.org/10.1016/j.pbiomolbio.2023.06.002>
- [7] Triantaphyllopoulos, E., & Triantaphyllopoulos, D. C. (1967). Amino acid composition of human fibrinogen and anticoagulant derivatives. *Biochemical Journal*, 105(1), 393–400. <https://doi.org/10.1042/bj1050393>
- [8] Matsuda, M., & Sugo, T. (2002). Structure and function of human fibrinogen inferred from dysfibrinogens. *International Journal of Hematology*, 76(Suppl 1), 352–360. <https://doi.org/10.1007/BF03165284>
- [9] Wolberg, A. S. (2023). Fibrinogen and fibrin: Synthesis, structure, and function in health and disease. *Journal of Thrombosis and Haemostasis*, 21(11), 3005–3015. <https://doi.org/10.1016/j.jtha.2023.08.014>
- [10] Benson, M. D., Buxbaum, J. N., Eisenberg, D. S., Merlini, G., Saraiva, M. J. M., Sekijima, Y., Sipe, J. D., Westermark, P., & International Society of Amyloidosis Nomenclature Committee. (2018). Amyloid nomenclature 2018: Recommendations by the International Society of Amyloidosis (ISA) nomenclature committee. *Amyloid*, 25(4), 215–219. <https://doi.org/10.1080/13506129.2018.1549825>
- [11] Ein, D., Kimura, S., Terry, W. D., Magnotta, J., & Glenner, G. G. (1972). Amino acid sequence of an amyloid fibril protein of unknown origin. *Journal of Biological Chemistry*, 247(17), 5653–5655.
- [12] University of Debrecen. (2024). Test report K24/153: Kjeldahl crude protein and amino-acid analysis (MSZ EN ISO 5983-2:2009; MSZ EN ISO 13903:2005). Supplementary Material A (certificate and chromatograms).
- [13] Buxbaum, J. N., Eisenberg, D. S., Fändrich, M., McPhail, E. D., Merlini, G., Saraiva, M. J. M., Sekijima, Y., Westermark, P., Sipe, J. D., Benson, M. D., & International Society of Amyloidosis Nomenclature Committee. (2024). Amyloid nomenclature 2024: Update, novel proteins, and recommendations by the International Society of Amyloidosis (ISA) Nomenclature Committee. *Amyloid*. Advance online publication. <https://doi.org/10.1080/13506129.2024.2405948>
- [14] Ząbczyk, M., Ariëns, R. A. S., & Undas, A. (2023). Fibrin clot properties in cardiovascular disease: From basic mechanisms to clinical practice. *Cardiovascular Research*, 119(1), 94–111. <https://doi.org/10.1093/cvr/cvad017>
- [15] Westman, H., Hammarström, P., & Nyström, S. (2025). SARS-CoV-2 spike protein amyloid fibrils impair fibrin formation and fibrinolysis. *Biochemistry*, 64(24), 4818–4829. <https://doi.org/10.1021/acs.biochem.5c00550>
- [16] Mörz, M., Donzelli, A., Clancy, R. L., Sano, S., Fukushima, M., & Polykretis, P. (2026). Detection of vaccine-derived spike protein associated with immune cell infiltration in the heart and liver: A report of two cases. *Cells*, 15(11), Article 978. <https://doi.org/10.3390/cells15110978>
- [17] Solarino, B., Ambrosi, L., Benevento, M. *et al.* Cadaver clots: a systematic review of the literature. *Forensic Sci Med Pathol* **21**, 1831–1842 (2025). <https://doi.org/10.1007/s12024-025-00976-y>
- [18] Kell, D. B., & Pretorius, E. (2017). Proteins behaving badly: Substoichiometric molecular control and amplification of the initiation and nature of amyloid fibril formation — lessons from and for blood clotting. *Progress in Biophysics and Molecular Biology*, 123, 16–41. <https://doi.org/10.1016/j.pbiomolbio.2016.08.006>
- [19] Kell, D. B., Khan, M. A., & Pretorius, E. (2024). Fibrinoid microclots in long COVID: Assessing the actual evidence properly. *Research and Practice in Thrombosis and Haemostasis*, 8(7), Article 102566. <https://doi.org/10.1016/j.rpth.2024.102566>
- [20] Kell, D. B., & Pretorius, E. (2024). Proteomic evidence for amyloidogenic cross-seeding in fibrinoid microclots. *International Journal of Molecular Sciences*,

25(19), Article 10809.
<https://doi.org/10.3390/ijms251910809>

- [21] Louros, N., Konstantoulea, K., De Vleeschouwer, M., Ramakers, M., Schymkowitz, J., & Rousseau, F. (2020). WALTZ-DB 2.0: an updated database containing structural information of experimentally determined amyloid-forming peptides. *Nucleic acids research*, 48(D1), D389–D393. <https://doi.org/10.1093/nar/gkz758>
- [22] Marx, J., Hudry-Clergeon, G., Capet-Antonini, F., & Bernard, L. (1979). Laser Raman spectroscopy study of bovine fibrinogen and fibrin. *Biochimica et biophysica acta*, 578(1), 107–115. [https://doi.org/10.1016/0005-2795\(79\)90118-1](https://doi.org/10.1016/0005-2795(79)90118-1)



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third-party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <https://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2026

Supplementary Material

Supplementary A



Institute for Solid State Physics and Optics
HUN-REN Wigner Research Centre for Physics

Report

on Raman measurements performed on intravascular fibrous specimens

Author: Miklós Veres, HUN-REN Wigner RCP

Measurement conditions

The Raman spectra were recorded on a Renishaw InVia micro-Raman spectrometer. 633 nm and 785 nm excitations were used for the measurements. 532 nm was also tried but it excited intense fluorescence background overlapping the Raman bands. A 50x microscope objective (NA = 0.9) was used to focus the excitation laser and to collect the scattered light. The excitation spot diameter was around 2-4 microns for both excitations. The laser power at the sample was cca. 5 mW for the 633 nm and cca. 7 mW for the 785 nm excitation. The spectra were recorded in the 200-3100 cm^{-1} (633 nm) and 200-3200 cm^{-1} (785 nm) regions. A 10 seconds integration time was used in both cases with 10x accumulation, meaning 100 seconds total collection time. During the processing of the spectra, a polynomial fit was used to subtract the fluorescence background of the spectra in the Renishaw WIRE software. If it was necessary, the built-in cosmic ray removal function of the software was used to remove the artefacts.

Samples

The Raman measurements were performed on 2 samples (S1 and S2) delivered in vials. The solid particles of a few mm size were removed from the liquid with tweezers and placed on a silicon wafer. The Raman measurements were performed after they completely dried.

The samples on the silicon wafer are shown in Figure 1. It can be seen that after drying the two samples have different color. While S1 has light brown- yellow color, S2 is dark brown. Perhaps a detailed optical image analysis of the dried samples, with special focus on the polarization properties could also contribute to the differentiation of the two samples.

5-5 Raman spectra were recorded with 633 and 785 nm excitations of the two samples. Photos of typical spectrum recording spots are shown in Figure 2. The light and dark shades of the S1 and S2 can be seen here as well.

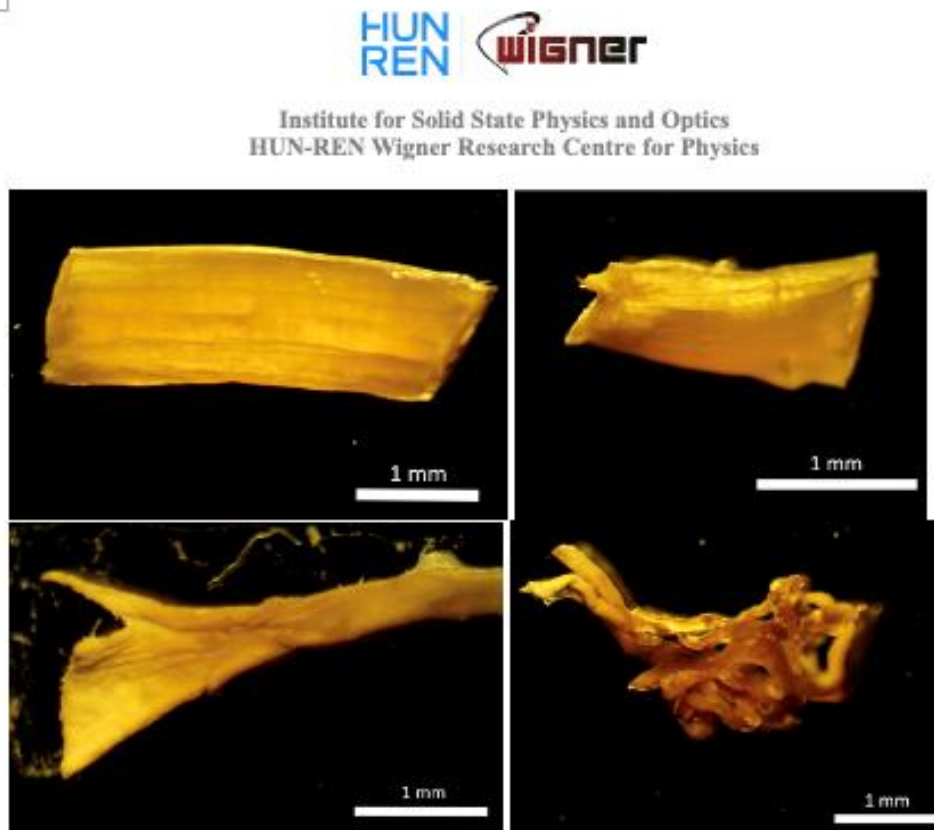


Figure 1. Samples S1 (top) and S2 (bottom) on the silicon wafer.



Institute for Solid State Physics and Optics
HUN-REN Wigner Research Centre for Physics

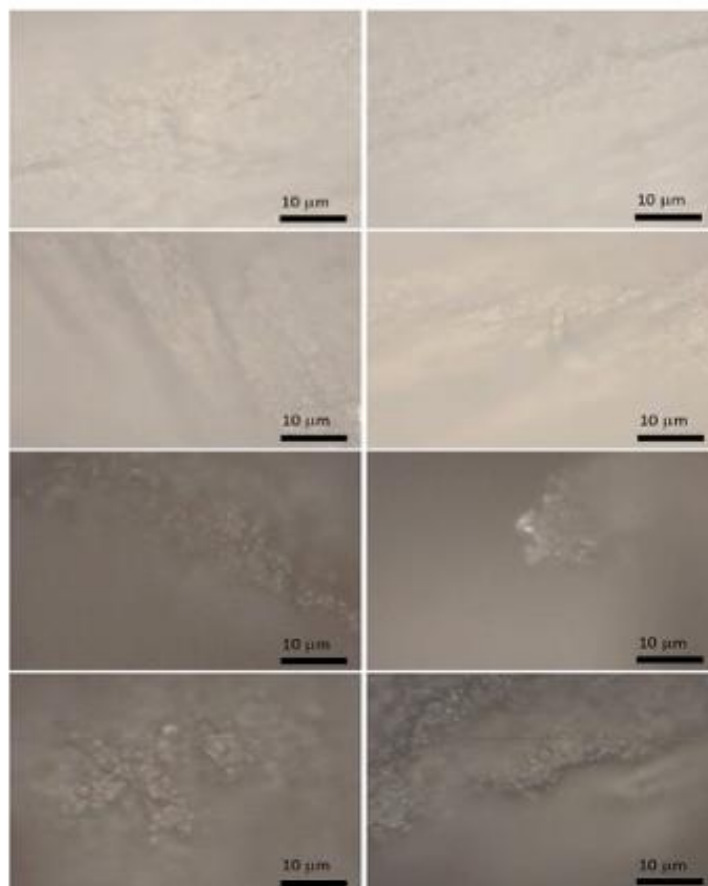


Figure 2. Typical morphology of the S1 and S2 samples around the measurement spots.

P. O. Box 49, 1525 Budapest, Hungary
Konkoly-Thege Miklós út 29-33, 1121 Budapest, Hungary
Phone: +36 1 392 2768 E-mail: wigner@wigner.hun-ren.hu
Web: wigner.hun-ren.hu

Raman results

The 633 nm excited Raman spectra of the S1 and S2 samples are shown in Figure 3. From the comparison of the two series of spectra, it can clearly be seen that bonding structure of the two samples is not identical. The S2 sample shows more strong Raman features compared to S1, and there are Raman peaks being present only in the spectra of one sample. Spectra recorded on different pieces of the same sample are similar.

The main features can be found in the 600-900, 1000-1700 and 2800-3100 cm^{-1} regions. Presence of the intense and composite Raman band in the latter region clearly indicates that both samples contain large amounts of organic matter. The Raman peaks at 2870, 2930, and 2970 cm^{-1} are related to symmetric and antisymmetric $\text{sp}^3 \text{CH}_2$ and $\text{sp}^3 \text{CH}_3$ stretching vibrations. The CH_2 stretching peak is shifted higher because the carbon is bound directly to the oxygen. These bands indicate the presence of methyl, methoxy/ethoxy groups in the samples. The 3060 cm^{-1} band, on the other hand, is related to $\text{sp}^2 \text{CH}$ stretching vibrations, meaning the presence of (substituted) aromatic rings in the samples.

The combination of the above identified functional groups could be assigned to aromatic polymers with methyl side-chains, toluene derivatives, or aromatic amino acids/proteins. Based on the background of the samples, the latter assignment is reasonable. Examples of such proteins are phenylalanine or tyrosine.

In the fingerprint region (1000-1700 cm^{-1}) the strongest Raman bands are at 1655, 1460, 1445, 1363, 1315, 1000 and 893 cm^{-1} (sample S1, 633 nm excitation), and at 1648, 1620, 1553, 1460, 1393, 1335, 1315, 1255, 1121, 1000, 753 cm^{-1} (sample S2, 633 nm excitation). Some of these peaks belong to the functional groups identified in the CH stretching region above. The peak around 1655 cm^{-1} can be assigned to Amide I, while the feature around 1250 cm^{-1} to Amide III vibrations. The sharp and strong band around 1000 cm^{-1} is the ring-breathing vibration of the substituted aromatic ring, and is a potential marker of Phenylalanine. The strong Raman peak around 752 cm^{-1} can be assigned to Tryptophan.

P. O. Box 49, 1525 Budapest, Hungary
Konkoly-Thege Miklós út 29-33, 1121 Budapest, Hungary
Phone: +36 1 392 2768 E-mail: wigner@wigner.hun-ren.hu
Web: wigner.hun-ren.hu



Institute for Solid State Physics and Optics
HUN-REN Wigner Research Centre for Physics

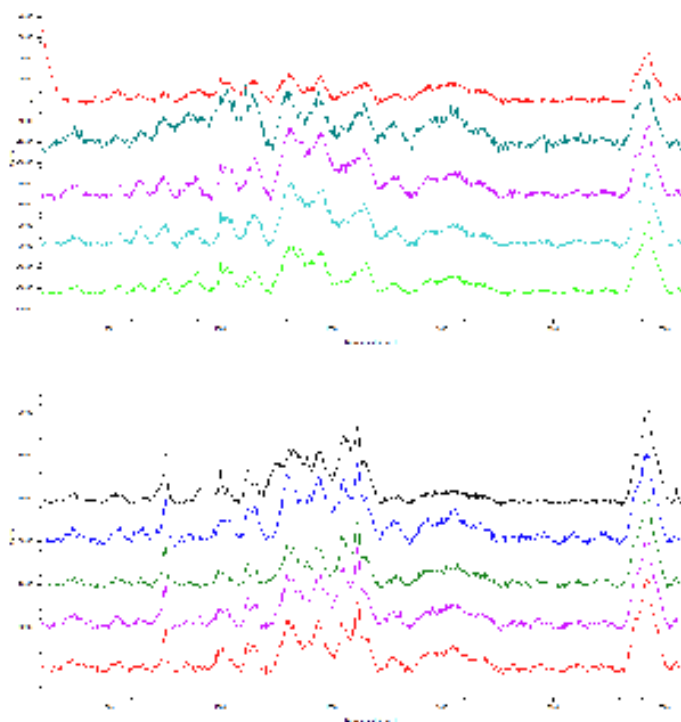
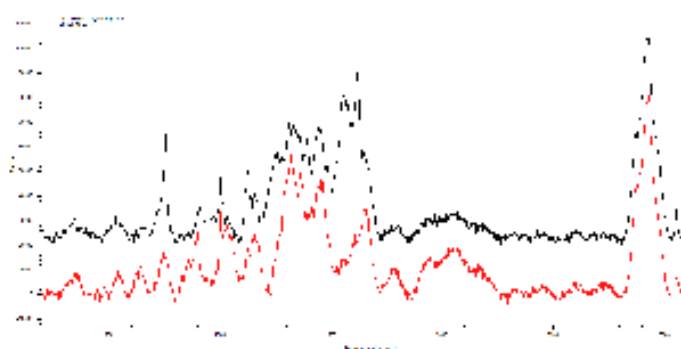


Figure 3. Series of Raman spectra recorded on the S1 (top) and S2 (bottom) samples with 633 nm excitation



P. O. Box 49, 1525 Budapest, Hungary
Konkoly-Thege Miklós út 29-33, 1121 Budapest, Hungary
Phone: +36 1 392 2768 E-mail: wigner@wigner.hun-ren.hu
Web: wigner.hun-ren.hu

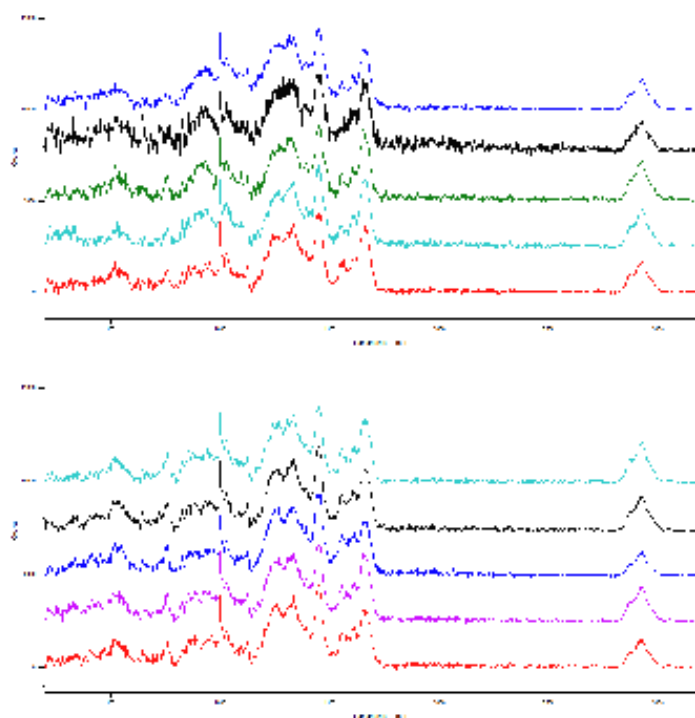


Institute for Solid State Physics and Optics
HUN-REN Wigner Research Centre for Physics

Figure 4. Comparison of typical 633 nm excited Raman spectra of the S1 (red) and S2 (black) samples.

The comparison of the typical Raman spectra of samples S1 and S2 is provided in Figure 4. The main differences are: the 750 cm^{-1} band (dominates in S2), 896 cm^{-1} (dominates S1), 1122 cm^{-1} (present in S2), 1365 cm^{-1} (S1), 1395 cm^{-1} (S2), 1621 cm^{-1} (S2). The Amide I/Amide III band intensity ratio is high in the S1 and low in the S2 sample, which indicates different stages of amyloid fibril formation.

The 785 nm excited Raman spectra of the samples are shown in Figure 5. The spectra show similar features to the 633 nm ones. There are no so remarkable differences between the spectra recorded on samples S1 and S2, as with the other excitation. The main reason for this could be the resonant Raman process, which amplifies the Raman peaks of specific constituents of the sample, if the excitation photon energy is equal or close to their existing electronic transitions. This is supported by the intensity of the benzene stretching vibration, being much weaker with 785 nm excitation. This means 633 nm excited the vibrations of related structural units resonantly.



P. O. Box 49, 1525 Budapest, Hungary
Konkoly-Thege Miklós út 29-33, 1121 Budapest, Hungary
Phone: +36 1 392 2768 E-mail: wigner@wigner.hun-ren.hu
Web: wigner.hun-ren.hu



Institute for Solid State Physics and Optics
HUN-REN Wigner Research Centre for Physics

Figure 5. Series of Raman spectra recorded on the S1 (top) and S2 (bottom) samples recorded with 785 nm excitation.

Comparison of the typical 785 nm excited spectra of the two samples is given in Figure 6. It can be seen that the curves are much more similar than the 633 nm excited spectra. The main differences are the more intense 752 cm^{-1} band in the S2 sample (similarly to the 633 nm case) and the higher Amide I/Amide III ratio on the S1 sample (similarly to the 633 nm case, but less pronounced).

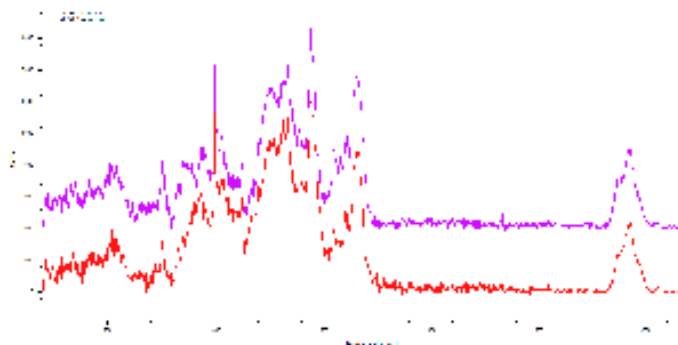


Figure 6. Comparison of typical 785 nm excited Raman spectra of the S1 (red) and S2 (purple) samples.

Discussion

The main Raman bands found in the spectra of the S1 and S2 samples, as well as their assignments are provided in Table 1.

Table 1. Main Raman bands found in the Raman spectra of the two samples and their assignment.

Raman peak position, cm ⁻¹		Assignment
S1 sample	S2 sample	
3060	3060	sp ² CH stretching (aromatic)
2970	2970	antisymmetric sp ³ CH ₃ stretching
2930	2930	antisymmetric sp ³ CH ₂ stretching
2870	2870	symmetric sp ³ CH ₃ stretching
1655	1648	Amide I (C=O stretch)
	1620	Amide I, intermolecular β -sheet (C=O stretch)
1550	1553	Amide II (N-H bend + C-N stretch)
1460	1450	sp ³ CH ₂ and sp ³ CH ₃ deformation
	1393	COO ⁻ symmetric stretch / CH ₃ deformation
1363		Tryptophan W7
	1335	CH deformation
1315	1315	CH ₂ twist
1256	1255	Amide III (N-H bend + C-N stretch)
	1121	C-N / C-O-P stretch
1030	1030	Phenylalanine deformation
1000	1000	Aromatic ring-breathing, phenylalanine
893		C-C stretch
744	753	Tryptophan W18
647	647	Tyrosine deformation
620	620	Phenylalanine deformation
545	545	S-S stretch

The detailed analysis of the C-H stretching region around 3000 cm⁻¹ indicates the presence of aromatic structural units, and different C-H functional groups. In protein-rich samples, these can be phenylalanine, tyrosine, tryptophan, or phenyl-containing molecules.

Protein misfolding. The fingerprint region is dominated by Amide I, II and III bands. The Amide I band at 1650 cm⁻¹ arises from α -helical C=O stretching and represents the native, pre-misfolding structural state, while the Amide III band at 1255 cm⁻¹ is associated with β -sheet or disordered backbone conformations. The high Amide I/Amide III ratio in S1 indicates that it retains a predominantly α -helical structure, consistent with a native-like or early intermediate state. The low ratio in S2 reflects a shift toward β -sheet dominance, indicating more advanced protein misfolding. This conclusion is further supported by the strong Amide I sub-band at 1620 cm⁻¹ in S2, which is a signature of intermolecular β -sheet formation characteristic of amyloid aggregation. Both samples contain both structural elements, but in different proportions, with β -



Institute for Solid State Physics and Optics
HUN-REN Wigner Research Centre for Physics

aggregation being dominant in S2. S2 has thus progressed further along the misfolding pathway, while S1 retains more native-like character.

Phosphorylation. The 1393 cm^{-1} , being present only in the S2 spectra, can be related to a higher degree of phosphorylation or more ordered phosphate–sidechain interactions. Combined with the sharper stronger 1620 cm^{-1} peak, this feature could indicate that phosphorylation has successfully locked the β -sheet geometry.

The 1121 cm^{-1} peak, being present in the spectrum of the S2 sample only, can also indicate the presence of phosphoester C–O–P modes from phosphoserine/phosphothreonine. Its presence is consistent with phosphorylation of the S2 sample.

According to the Raman spectra, S2 shows the spectral signature of a phosphorylation-stabilized amyloid, while S1 appears to be in a state where phosphorylation is incomplete.

Sulfur Sequestration.

The 545 cm^{-1} S–S stretch is visible in both spectra, confirming disulfide bonds persist in both samples.

This peak is conformationally sensitive, and since both samples show the band near 545 cm^{-1} , it can be concluded that the same disulfide rotamer is present in both samples.

Therefore, the disulfide geometry is dictated by the protein fold rather than by the aggregation state, since the S–S linkage occupies the same local conformation for both samples.

The high intensity of the band indicates that sulfur is structurally integrated within the fiber matrix in both cases.

P. O. Box 49, 1525 Budapest, Hungary
Konkoly-Thege Miklós út 29-33, 1121 Budapest, Hungary
Phone: +36 1 392 2768 E-mail: wigner@wigner.hun-ren.hu
Web: wigner.hun-ren.hu



Institute for Solid State Physics and Optics
HUN-REN Wigner Research Centre for Physics

The tryptophan W18 band around 750 cm^{-1} reports on the indole ring breathing mode, whose intensity is sensitive to the local dielectric and to electron-transfer quenching by nearby sulfur. The Raman intensity of this band relative to the 1000 cm^{-1} phenylalanine internal standard is much smaller in the spectrum of the S1 sample. This suggests that tryptophan is in closer proximity to sulfur in that sample, i.e. there is a more effective sulfur sequestration into the aromatic core.

Waltz Sequence Recruitment: Spectroscopic signatures of aromatic amino acids (phenylalanine ($1000, 1030, 622\text{ cm}^{-1}$), tryptophan ($753, 1363\text{ cm}^{-1}$), tyrosine (647 cm^{-1})) can be found in both samples. This could indicate the presence of Waltz-like aromatic-rich sequences.

The width of the 1000 cm^{-1} phenylalanine ring breathing mode directly reports on the conformational homogeneity of phenylalanine environments. In a Waltz-stacked amyloid core this band is narrow and sharp (S2 sample), while in a disordered or partially assembled state, phenylalanine rings occupy multiple orientations, broadening the peak (S1 sample). The relative intensity of the 753 (tryptophan) and 1000 cm^{-1} (phenylalanine) peaks shows remarkably higher tryptophan content in the S2 sample. The same analysis of the tyrosine peak around 647 cm^{-1} indicates higher tyrosine content in S1.

Summary

The Raman spectra of the two samples reveal distinct stages along the amyloid formation pathway. S1 presents spectral characteristics of a native-like, pre-fibrillar protein, while S2 exhibits the hallmarks of a structurally mature amyloidogenic assembly.

Two complementary metrics support this assignment: structural order, as read from secondary structure markers, and spectral definition, as reflected in peak sharpness and resolution.

The Amide I/Amide III intensity ratio distinguishes the two states. S1 is dominated by the 1650 cm^{-1} α -helical Amide I band, retaining the native backbone fold. In S2, this ratio shifts decisively: the 1255 cm^{-1} Amide III β -sheet component strengthens and a prominent 1620 cm^{-1} sub-band emerges — the spectroscopic signature of intermolecular β -sheet formation characteristic of amyloid cross- β architecture. This shift from α -helical to β -sheet dominance marks the structural transition from a biologically flexible protein to a misfolded, aggregation-prone conformer.

The spectral definition changes in parallel. In S1, the 1000 cm^{-1} phenylalanine ring breathing mode is broad, reflecting aromatic residues sampling multiple orientations in a conformationally flexible environment. In S2, this same band sharpens markedly, indicating that phenylalanine rings have been locked into uniform, repeating positions — consistent with ordered π -stacking within a WALTZ-like aromatic core. The presence of tryptophan ($753, 1363\text{ cm}^{-1}$), tyrosine (647 cm^{-1}), and phenylalanine ($1000, 1030, 622\text{ cm}^{-1}$) markers in both samples confirms the aromatic-rich composition required for such stacking-driven self-assembly.

P. O. Box 49, 1525 Budapest, Hungary
Konkoly-Thege Miklós út 29-33, 1121 Budapest, Hungary
Phone: +36 1 392 2768 E-mail: wigner@wigner.hun-ren.hu
Web: wigner.hun-ren.hu



Institute for Solid State Physics and Optics
HUN-REN Wigner Research Centre for Physics

Additional spectral features exclusive to S2 reinforce the picture of a stabilized scaffold. The 1393 cm^{-1} and 1121 cm^{-1} bands, absent in S1, are consistent with phosphorylation of serine or threonine residues, suggesting that post-translational modification contributes to locking the β -sheet geometry in the mature state.

The disulfide S–S stretch at 545 cm^{-1} persists in both samples in the same ggG rotameric conformation, demonstrating that covalent cross-linking is established early and maintained throughout the transition, providing a structural anchor that is independent of the aggregation state.

The transformation from S1 to S2 is therefore not simple denaturation. Denatured protein would produce broad, featureless Raman bands characteristic of a random coil. Instead, S2 displays the opposite: narrow, well-resolved peaks indicative of a rigid, highly ordered molecular assembly. The proteins have not merely lost their native fold — they have adopted a new, thermodynamically stable architecture reinforced by backbone hydrogen bonding, aromatic packing, electrostatic phosphorylation contacts, and covalent disulfide bridges. This combination of stabilizing interactions is consistent with a persistent, self-assembling amyloidogenic scaffold rather than a reversible aggregation event.



**DEBRECENI
EGYETEM**

Mezőgazdaság-, Élelmiszertudományi
és Környezetgazdálkodási Kar Agrárműszerközpont
H-4032 Debrecen, Bösztörményi út 138.
H-4002, Debrecen Pf.: 400
Tel/fax: 52/526-980, email: pusztahelyi@agr.unideb.hu

Iktatószám: KE153/2024

Dátum: 2024.04.16.

oldal 1 / 2

VIZSGÁLATI JEGYZŐKÖNYV

Ügyfél adatai:

Beküldő neve: File Márk
Címe: 2600 Vác, Zöldfa u. 28.

A minta adatai

Minta azonosító: K24/153

Minta megnevezése: 1 db fehérje minta

Mintavétel: ☐ Laboratórium vette a mintát ☒ Megrendelő vette a mintát

☐ akkreditált ☒ nem akkreditált

Vizsgálat költsége: 25 300Ft+ÁFA=32 131Ft

Mintavétel ideje:	Beérkezés ideje:	Vizsgálat kezdete:	Vizsgálat vége:
nem ismert	2024.04.08.	2024.04.08.	2024.04.16.

Vizsgált paraméter (mértékegység)	Vizsgálati módszer	Megengedett vizsgálati eltérés
Nyersfehérje (m/m)% Kjeldahl - módszer	MSZ EN ISO 5983-2:2009	± 0,60 m/m%
Aminosav-összetétel (m/m%) Ioncserés kromatográfia	MSZ EN ISO 13903:2005	±15%R

Vizsgált paraméter (mértékegység)	K24/153 fehérje minta
Minta megnevezése	55708 G
Nyersfehérje (m/m)% Kjeldahl - módszer	7,72





**DEBRECENI
EGYETEM**

Mezőgazdaság-, Élelmiszertudományi
és Környezetgazdálkodási Kar Agrárműszerközpont
H-4032 Debrecen, Böszörményi út 138.
H-4002, Debrecen Pf.: 400
Tel/fax: 52/526-980, email: pusztahelyi@agr.unideb.hu

Iktatószám: KE153/2024

Dátum: 2024.04.16.

oldal 2 / 2

Vizsgált paraméter (mértékegység)	K24-153 fehérje minta
Aminosav-összetétel (m/m%)	55708 G
ASP	1,19
THR	0,27
SER	0,58
GLU	0,60
PRO	1,55
GLY	0,37
ALA	0,21
CYS	0,01
VAL	0,20
MET	0,05
ILE	0,16
LEU	0,33
TYR	0,05
PHE	0,07
HIS	0,22
LYS	1,18
ARG	0,26

Az eredmények eredeti anyagra vonatkoznak.

A vizsgálati jegyzőkönyv 2 db számozott oldalt tartalmaz.

A vizsgálati eredmények csak a megvizsgált mintára vonatkoznak.

Az eredményekkel kapcsolatos véleményt vagy értékelést a laboratórium nem adhat ki.

A vizsgálati jelentést a vizsgáló laboratórium engedélye nélkül csak teljes terjedelmében szabad lemásolni.

Reklamációt az eredmény kiadását követően 10 napon belül fogadunk el.

Debrecen, 2024.04.16.

Kovács Tünde

Prof. Dr. Pusztahelyi Tünde
egyetemi tanár, agrárműszerközpont vezető

