COURSE DESCRIPTION

Characterization of Transient Species in Chemistry and Biochemistry

Academic year 2025-2026

1. Programme-related data

1.1. Higher Education Institution	Babeș-Bolyai University, Cluj-Napoca
1.2. Faculty	Chemistry and Chemical Engineering
1.3. Department	Doctoral School of Chemistry
1.4. Field	Chemistry
1.5. Level of study	Doctorate
1.6. Degree programme / Qualification	Doctorate / Doctor in Chemistry
1.7. Form of education	Full time

2. Course-related data

2.1. Course title	***Characterization of Transient Species in Chemistry and Biochemistry***			Course code	**SDC-19-12**
2.2. Course coordinator	Prof. Dr. Radu Lucian SILAGHI-DUMITRESCU				
2.3. Seminar coordinator	Prof. Dr. Radu Lucian SILAGHI-DUMITRESCU				
2.4. Year of study	**1**	2.5. Semester	**2**	2.6. Type of assessment	**Exam**
2.7. Course status	**Optional**	2.8. Course type	**Specialisation subject**		

3. Total estimated time (hours per semester of teaching activities)

3.1. Number of hours per week	**3**	of which: 3.2. course	**2**	3.3. seminar/ laboratory/ project	**1**
3.4. Total of hours in the curriculum	**36**	of which: 3.5. course	**24**	3.6. seminar/ laboratory	**12**

Time allocation for individual study (IS) and self-taught activities (ST)	**hours**
Learning from textbooks, course materials, bibliography, and notes (IS)	60
Additional research in the library, on subject-specific electronic platforms, and on-site	60
Preparing seminars/ laboratories/ projects, assignments, reports, portfolios, and essays	50
Tutoring (professional guidance)	20
Examinations	20
Other activities	4
3.7. Total hours of individual study (IS) and self-taught activities (ST)	**214**
3.8. Total hours per semester	**250**
3.9. Number of credits	**10**

4. Prerequisites (where applicable)

4.1. curriculum-related	Master's-level training in chemistry, biochemistry, or a related discipline, including undergraduate physical chemistry and spectroscopy.
4.2. skills-related	Familiarity with basic reaction kinetics and with the main spectroscopic techniques (UV-Vis, IR, NMR, EPR); reading-level English.

5. Specific conditions (where applicable)

5.1. course-related	Students attend lectures having read the assigned material beforehand; mobile phones off or silent; active participation in the mechanistic discussions.
5.2. seminar/laboratory-related	Students attend the seminar prepared with notes and the week's assigned primary literature; active, evidence-based participation in kinetic-data and mechanism workshops.

6.1. Competencies resulting from the completion of the degree programme

Professional competencies	
Competency code	**Competency**
PC1	Definition of notions, concepts, theories and advanced models in the field of Chemistry as well as their proper use within the professional community
PC4	Use of in-depth knowledge of chemistry to explain and interpret tools and processes specific to the detection and characterization of transient species and reaction intermediates
PC5	Identifying and applying advanced concepts, methods and theories to solve problems specific to the kinetic and spectroscopic characterization of reactive intermediates in chemistry and biochemistry
Transversal competencies	
Competency code	**Competency**
TC2	Execution of the requested tasks according to the specified requirements and within the imposed deadlines, in compliance with the norms of professional ethics and moral conduct, following a pre-established work plan
TC3	Solving the requested tasks in accordance with the general objectives established by integration within a working group

6.2. Learning outcomes relevant to the degree programme (as referred to in the curriculum)

Learning outcomes targeted by the subject		
Competency code	**Knowledge and comprehension**	**Specific academic skills**
PC1	Advanced and up-to-date knowledge in chemistry and biochemistry, with emphasis on reaction mechanisms and the nature of transient species (radicals, carbenes, carbocations/anions, excited states, enzyme intermediates); critical understanding of the primary literature on reactive intermediates.	Applies and synthesizes advanced concepts to formulate mechanistic hypotheses and to design characterization strategies; justifies the choice of spectroscopic and kinetic approaches.
PC4	Advanced research methodologies: stopped-flow, rapid-freeze quench, pulse radiolysis, flash photolysis, time-resolved UV-Vis/IR/Raman, EPR and pulsed EPR, CIDNP/PHIP, matrix isolation, online MS, time-resolved X-ray	Designs, plans and implements a characterization experiment targeting a transient species; applies quality and safety procedures and documents stages and data with full traceability.

	crystallography and cryo-EM; principles of research project management.	
PC5	Advanced strategies for data analysis, interpretation, and integration of kinetic traces and spectroscopic spectra (global analysis, target analysis, spectral deconvolution); evaluation of methodological limitations, reproducibility, and scientific impact.	Critically analyses complex time-resolved data, estimates uncertainty and formulates mechanistic conclusions; proposes methodological improvements and directions for research/innovation.
TC2	Scientific terminology and communication in Romanian and English; the structure of scientific articles and presentations in mechanistic chemistry/biochemistry; good citation practices.	Writes and presents scientific results in Romanian/English coherently; provides well-argued responses to comments and peer reviews.
TC3	Digital tools for research, citation, and data processing (kinetic-fitting packages, spectroscopy processing software, reference managers, structural databases); responsible use of digital resources.	Uses discipline-specific digital tools effectively; manages citations; uses digital resources responsibly in research activities.

7. Subject-specific learning outcomes

Knowledge and comprehension
1. Applying elementary kinetic frameworks (rate laws, pre-equilibrium, steady-state, Curtin-Hammett, kinetic isotope effects) to classify and analyse transient intermediates in chemical and enzymatic reactions
2. Understanding and interpreting time-resolved spectroscopic data (UV-Vis, IR, Raman, EPR, NMR, MS, X-ray diffraction) for the identification of short-lived species
3. Critical analysis and use of advanced methods (stopped-flow, rapid-freeze quench, flash photolysis, pulse radiolysis, matrix isolation) for the quantitative and qualitative evaluation of intermediate populations and rate constants
4. Awareness of key case studies in biochemistry (cytochrome P450 Compound I, ribonucleotide-reductase tyrosyl radical, photosystem II oxygen-evolving complex, myoglobin-O2/CO) and in organometallic/organic chemistry (carbenes, nitrenes, radical-pair intermediates)
Specific academic skills
1. Execution of the requested tasks according to specified requirements and within the imposed deadlines, in compliance with professional ethics and moral conduct, following a pre-established work plan
2. Concern for improving the results of the professional activity by active engagement in the characterization workshops and by peer-review of classmates' analyses
3. Ability to prepare written reports and to publicly defend/support these reports

8. Contents

8.1. Course		
Course/ Seminar topic	**Teaching and learning methods**	**Remarks**
8.1.1. Introduction: transient species and reaction intermediates in chemistry and biochemistry; classification (radicals, carbenes, nitrenes, carbocations/anions, excited states, enzyme intermediates); lifetimes from fs to minutes; why and how we characterize them.	Presentation; Explanation; Conversation; Description; Debate	

8.1.2. Kinetic frameworks for mechanistic analysis: elementary rate laws, pre-equilibrium and steady-state approximations, the Curtin-Hammett principle; kinetic isotope effects (KIE) as mechanistic probes; Hammett and Marcus analyses.	Presentation; Explanation; Conversation; Description; Debate	
8.1.3. Rapid-mixing methods: stopped-flow (photometric and fluorescence detection), continuous-flow, rapid chemical quench, rapid-freeze quench for EPR and Mössbauer; dead-time, mixing efficiency, and design considerations.	Presentation; Explanation; Conversation; Description; Debate	
8.1.4. Pulse radiolysis and flash photolysis: generating reactive intermediates photochemically and radiolytically; coupled spectroscopic detection; the classic Norrish–Porter experiments and modern laser flash photolysis.	Presentation; Explanation; Conversation; Description; Debate	
8.1.5. Time-resolved optical spectroscopy (UV-Vis): femto-, pico-, nano- and microsecond pump-probe methods; global and target analysis; spectral deconvolution of overlapping transients.	Presentation; Explanation; Conversation; Description; Debate	
8.1.6. Time-resolved vibrational spectroscopy: transient IR, transient Raman and resonance-enhanced Raman for the detection of bond-specific transients, including metallo-enzyme chromophoric intermediates.	Presentation; Explanation; Conversation; Description; Debate	
8.1.7. EPR spectroscopy of paramagnetic transients: continuous-wave and pulsed EPR; HYSCORE and ENDOR; spin-trapping with DMPO/PBN for short-lived radicals; Mössbauer spectroscopy of iron-containing intermediates.	Presentation; Explanation; Conversation; Description; Debate	
8.1.8. Transient NMR, CIDNP and parahydrogen-induced polarization (PHIP) for detecting radical-pair intermediates; freeze-quench NMR approaches; low-field NMR for in-situ monitoring.	Presentation; Explanation; Conversation; Description; Debate	
8.1.9. Matrix isolation and cryogenic trapping of reactive intermediates: noble-gas and N_2 matrices; low-temperature IR/UV/EPR; isolation of carbenes, nitrenes, and open-shell transition-metal species.	Presentation; Explanation; Conversation; Description; Debate	
8.1.10. Mass-spectrometric detection of transient species: on-line ESI-MS and cryo-MS of reaction intermediates; hydrogen-deuterium exchange mass spectrometry (HDX-MS) for protein conformational intermediates.	Presentation; Explanation; Conversation; Description; Debate	
8.1.11. Time-resolved X-ray crystallography (Laue diffraction, serial femtosecond crystallography at XFELs) and cryo-EM snapshots; XAS/EXAFS of metallo-enzyme intermediates; integrating structural with spectroscopic data.	Presentation; Explanation; Conversation; Description; Debate	
8.1.12. Integrated case studies with computational (DFT, QM/MM) support: cytochrome P450 Compound I, the oxygen-evolving complex of photosystem II, ribonucleotide-reductase tyrosyl radical, carbene/nitrene intermediates in transition-metal catalysis.	Presentation; Explanation; Conversation; Description; Debate	

Bibliography

1. Johnson, K. A. (2003). Kinetic Analysis of Macromolecules: A Practical Approach. Oxford University Press.

2. Fersht, A. (1999). Structure and Mechanism in Protein Science: A Guide to Enzyme Catalysis and Protein Folding. W. H. Freeman.

3. Moss, R. A., Platz, M. S., Jones, M. (Eds.) (2004). Reactive Intermediate Chemistry. Wiley.

4. Platz, M. S., Moss, R. A., Jones, M. (Eds.) (2007). Reviews of Reactive Intermediate Chemistry. Wiley.

5. Lakowicz, J. R. (2006). Principles of Fluorescence Spectroscopy, 3rd ed. Springer.

6. Weil, J. A., Bolton, J. R. (2007). Electron Paramagnetic Resonance: Elementary Theory and Practical Applications, 2nd ed. Wiley.

7. Hore, P. J. (2015). Nuclear Magnetic Resonance, 2nd ed. Oxford University Press.

8. Gütlich, P., Bill, E., Trautwein, A. X. (2011). Mössbauer Spectroscopy and Transition Metal Chemistry. Springer.

9. Bollinger, J. M., Krebs, C. (2006). "Stalking intermediates in oxygen activation by iron enzymes: motivation and method." J. Inorg. Biochem. 100, 586-605.

10. Rittle, J., Green, M. T. (2010). "Cytochrome P450 Compound I: capture, characterization, and C-H bond activation kinetics." Science 330, 933-937.

11. Schlichting, I., Berendzen, J., Chu, K., et al. (2000). "The catalytic pathway of cytochrome P450cam at atomic resolution." Science 287, 1615-1622.

12. Stubbe, J., van der Donk, W. A. (1998). "Protein radicals in enzyme catalysis." Chem. Rev. 98, 705-762.

13. Bailey, S. W., Fox, D. T. (2013). Biophysical Techniques in Photosynthesis. Kluwer.

14. Chance, B. (1991). "Optical method." Annu. Rev. Biophys. Biophys. Chem. 20, 1-28 (classic stopped-flow review).

8.2. Seminar/ laboratory

Course/ Seminar topic	**Teaching and learning methods**	**Remarks**
8.2.1. Landmark-paper workshop: how was cytochrome P450 Compound I identified and characterized? Close reading of Rittle & Green (Science, 2010) and precursor studies.	Conversation; Learning by discovery; Problem solving; Critical reading	
8.2.2. Stopped-flow data workshop: pseudo-first-order analysis of a biphasic kinetic trace; extracting k_obs, identifying concerted vs. stepwise pathways.	Conversation; Learning by discovery; Problem solving; Critical reading	
8.2.3. Rapid-freeze EPR: close reading of Bollinger & Stubbe on the ribonucleotide-reductase radical intermediate; recognizing transient EPR signatures in a time-course.	Conversation; Learning by discovery; Problem solving; Critical reading	
8.2.4. Flash photolysis in its classical form: reading Norrish & Porter (Nature, 1949/1950); reconstructing the kinetic analysis of the first recorded transient traces.	Conversation; Learning by discovery; Problem solving; Critical reading	
8.2.5. Ultrafast pump-probe workshop: distinguishing solvent heating/coherent artefacts from genuine photoproduct signals in a transient-absorption spectrum.	Conversation; Learning by discovery; Problem solving; Critical reading	
8.2.6. Time-resolved vibrational spectroscopy case: ligand rebinding to myoglobin (Austin & Frauenfelder, Biochemistry, 1975); CO-rebinding kinetics and geminate pairs.	Conversation; Learning by discovery; Problem solving; Critical reading	
8.2.7. Spin-trapping exercise: reading a reactive-oxygen-species paper and identifying the signature DMPO-OH/DMPO-OOH EPR patterns.	Conversation; Learning by discovery; Problem solving; Critical reading	
8.2.8. Matrix isolation classic: close reading of a paper on methylene (CH_2) or another carbene in a noble-gas matrix; what did the low-temperature IR actually prove?	Conversation; Learning by discovery; Problem solving; Critical reading	
8.2.9. Online-MS of enzyme intermediates: reading a study that uses ESI-MS to monitor a catalytic cycle; identifying transient m/z signals and quantifying populations.	Conversation; Learning by discovery; Problem solving; Critical reading	
8.2.10. Time-resolved diffraction: reading a paper on serial femtosecond crystallography of a transient state (e.g., Schlichting et al. on photolysed carbonmonoxy-myoglobin).	Conversation; Learning by discovery; Problem solving; Critical reading	

8.2.11. Computational support: reading a DFT/QM-MM study that corroborates the assignment of a transient species; evaluating the agreement between theory and spectroscopy.	Conversation; Learning by discovery; Problem solving; Critical reading	
8.2.12. Final presentations: students defend an integrated critical-analysis essay on one chosen transient species or case study, responding to questions and counter-arguments.	Conversation; Learning by discovery; Problem solving; Critical reading	

Bibliography

1. Norrish, R. G. W., Porter, G. (1949). "Chemical reactions produced by very high light intensities." Nature 164, 658.

2. Austin, R. H., Beeson, K. W., Eisenstein, L., Frauenfelder, H., Gunsalus, I. C. (1975). "Dynamics of ligand binding to myoglobin." Biochemistry 14, 5355-5373.

3. Groves, J. T., McClusky, G. A. (1976). "Aliphatic hydroxylation via oxygen rebound. Oxygen transfer catalyzed by iron." J. Am. Chem. Soc. 98, 859-861.

4. Bollinger, J. M., Edmondson, D. E., Huynh, B. H., Filley, J., Norton, J. R., Stubbe, J. (1991). "Mechanism of assembly of the tyrosyl radical-dinuclear iron cluster cofactor of ribonucleotide reductase." Science 253, 292-298.

5. Rittle, J., Green, M. T. (2010). "Cytochrome P450 Compound I: capture, characterization, and C-H bond activation kinetics." Science 330, 933-937.

6. Schlichting, I., Almo, S. C., Rapp, G., et al. (1994). "Time-resolved X-ray crystallographic study of the conformational change in Ha-Ras p21 protein on GTP hydrolysis." Nature 345, 309-315.

7. Janata, E., Schuler, R. H. (1982). "Rate constant for scavenging e-aq in N2O-saturated solutions." J. Phys. Chem. 86, 2078-2084 (pulse-radiolysis methodology).

8. Sension, R. J., Repinec, S. T., Szarka, A. Z., Hochstrasser, R. M. (1993). "Femtosecond laser studies of the cis-stilbene photoisomerization." J. Chem. Phys. 98, 6291.

9. Milne, C. J., Penfold, T. J., Chergui, M. (2014). "Recent experimental and theoretical developments in time-resolved X-ray spectroscopies." Coord. Chem. Rev. 277-278, 44-68.

10. Kern, J., Chatterjee, R., Young, I. D., et al. (2018). "Structures of the intermediates of Kok's photosynthetic water oxidation clock." Nature 563, 421-425 (SFX at XFEL).

9. Evaluation

Type of activity	9.1 Evaluation criteria	9.2 Evaluation methods	9.3 Percentage in the final grade
9.4. Course	Correctness of answers — proper understanding and learning of notions and concepts discussed during lectures; correct use of learned concepts within new contexts. Correct solving of the problems as part of the examination subjects.	Written report with critical analysis of a chosen case	**50%**
9.5. Seminar/ laboratory	Correctness of answers — proper understanding and learning of notions and concepts discussed during seminars; correct use of learned concepts within new contexts. Quality of reports, and quality of the final oral defense.	Final oral colloquium with defense of rebuttal essay	**50%**

9.6 Minimum standard for passing

Adequate knowledge of at least one case study per major technique family (rapid mixing; flash photolysis / pulse radiolysis; time-resolved optical spectroscopy; time-resolved vibrational spectroscopy; EPR / Mössbauer; time-resolved diffraction / XAS; mass spectrometry of intermediates); demonstrated ability to critically read and defend a primary paper reporting a transient-species characterization.

10. SDG labels (Sustainable Development Goals)

(Select a single SDG label that best matches the subject; if none applies, select "No label applies". The Quality Education label has been selected for this course.)

☐ **1** No Poverty	☐ **2** Zero Hunger	☐ **3** Good Health and Well-being	☒ **4** Quality Education	☐ **5** Gender Equality	☐ **6** Clean Water and Sanitation	☐ **7** Affordable and Clean Energy	☐ **8** Decent Work and Economic Growth	☐ **9** Industry, Innovation and Infrastructure
☐ **10** Reduced Inequality	☐ **11** Sustainable Cities and Communities	☐ **12** Responsible Consumption and Production	☐ **13** Climate Action	☐ **14** Life Below Water	☐ **15** Life on Land	☐ **16** Peace, Justice and Strong Institutions	☐ **17** Partnerships for the Goals	☐ — No label applies

Date of entry: 21.04.2026	**Signature of course coordinator** …… *Prof. Dr. Radu Lucian SILAGHI-DUMITRESCU*	**Signature of seminar coordinator** …… *Prof. Dr. Radu Lucian SILAGHI-DUMITRESCU*
Date of approval in CDSC: ……	**Signature of the Director of the DSC** …… *Prof. dr. Niculina Daniela Hădade*	