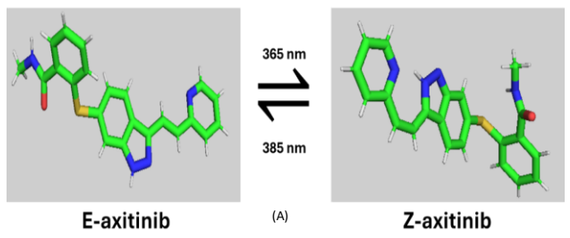
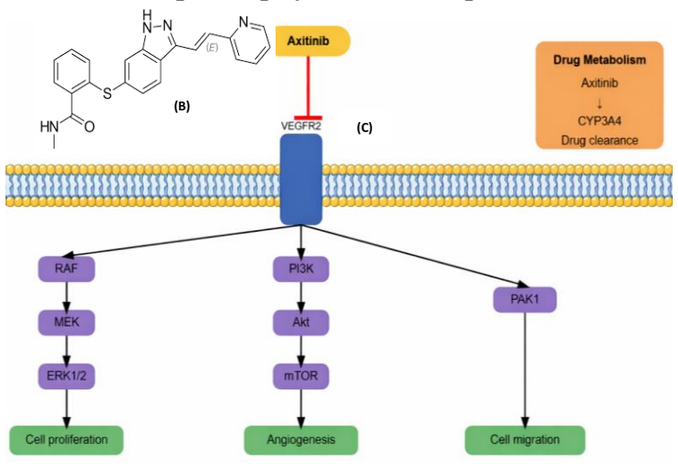
Ultrafast Photophysics of Axitinib by Transient Absorption Spectroscopy and Femtosecond Stimulated Raman Spectroscopy

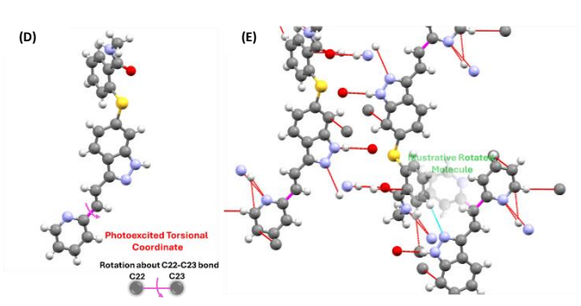
Atripan Mukherjee[a], Andrej Hovan[a], Jakub Dostal[a], Miroslav Kloz[a], Jonatan Johannesson[b], Gabriel Žoldák[b], Gustavo Fuertes[c] and Krishna Khakurel[a]
[a]Extreme Light Infrastructure ERIC, Dolni Brezany CZ-25241, Czech Republic
[b]Faculty of Science, Faculty of Science, Pavol Jozef Šafárik University in Košice, Park Angelinum 19, Košice 040 01, Slovakia
[c]Institute of Biotechnology of the Czech Academy of Sciences, Vestec CZ-25250, Czech Republic

Introduction

- Small-molecule tyrosine kinase inhibitor and approved for renal cell carcinoma
- Molecular photochemistry translates into structural response in molecular solids, remains a fundamental challenge[1,2]
- Chemical class: indazole-derived heteroaromatic compound.
- Molecular formula: $C_{22}H_{18}N_4OS$
- Exhibits light sensitivity → relevance to photochemistry
- Extended π-conjugation system
- Heteroaromatic rings facilitate photoexcitation
- Poor aqueous solubility, lipophilic character
- Absorption of UV/visible light
- Photoswitches, E-Z or(cis-trans)
- E-Z isomerisation is reported in the liquid state
- Transient photo physics is unexplored





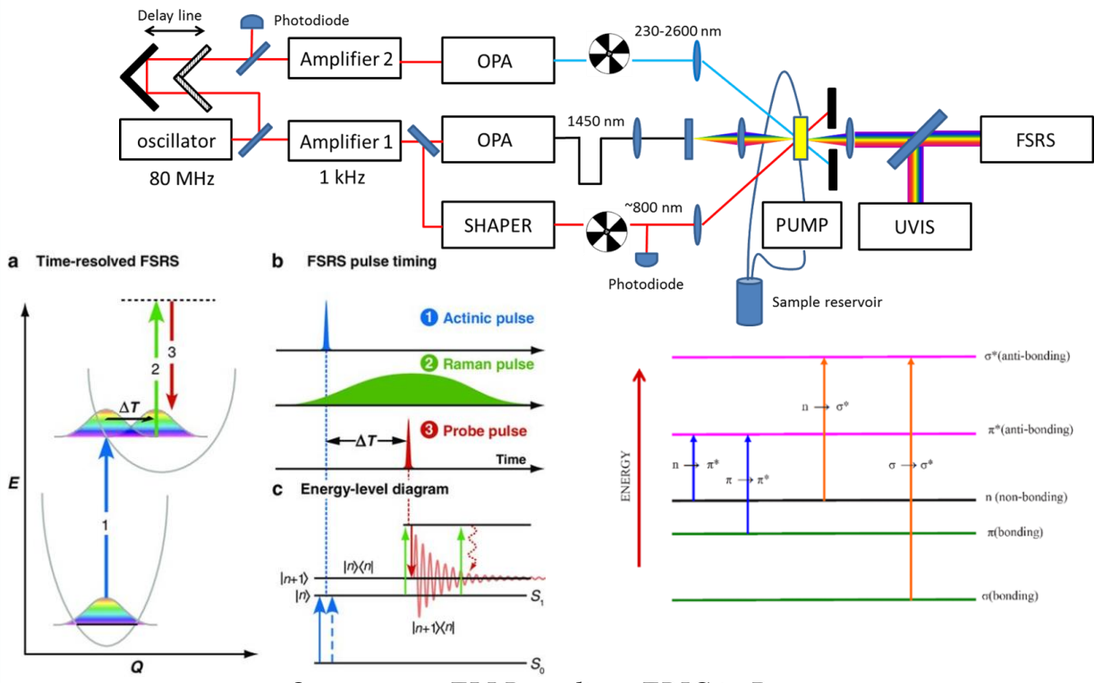


(A) Schematic of cis-trans isomerization of axitinib.
(B) Molecular structure of axitinib.

(C) This schematic illustrates inhibition of VEGFR2 signalling by axitinib at the endothelial cell membrane, with an expanded phospholipid bilayer representation to emphasisehe membrane environment.
(D) Molecular structure of axitinib highlighting the photoactive torsional coordinate.
(E) Local crystal packing showing the intermolecular environment around the reactive unit. Selected short contacts (red dashed lines) indicate steric constraints. A rotated geometry (transparent) illustrates that photoinduced torsion leads to unfavorable close contacts, requiring lattice distortion.

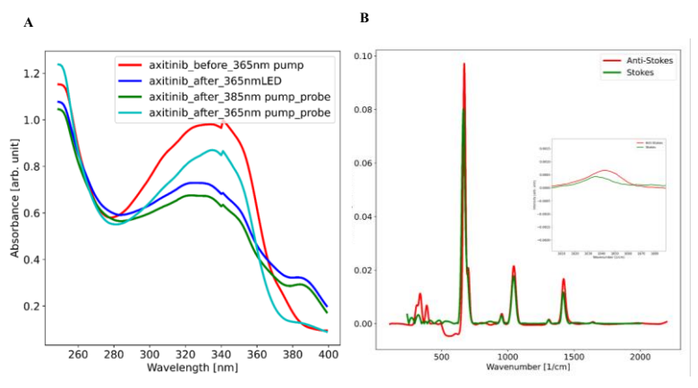
FSRS

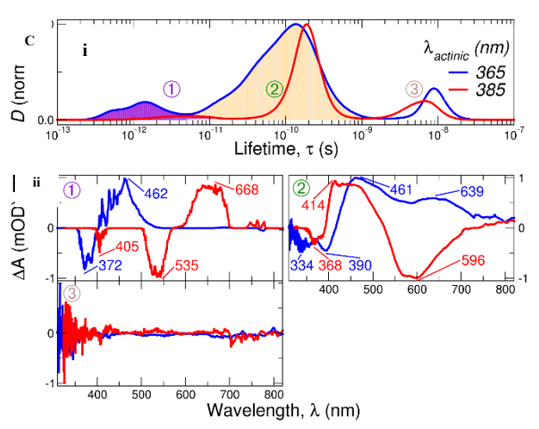
- FSRS method to gain detailed molecular information on photopharmacology reaction dynamics through recording of transient vibrational spectra[3].
- For photoreaction, 120 nJ and 1 µJ for 355 nm and 400nm, respectively from optical parametric amplifier (OPA) driven by Solstice amplifier focused into a 100 µm spot as actinic pump with pulse duration 50 fs[4].
- Signal beam of 1450 nm from second OPA system on CaF_2 plate to generate a white light supercontinum as the probe, focused on the sample with a spot size of 50 µm
- 800 nm fs pulses from the second amplifier passed through home-built pulse shaper to create a series of frequency-locked ps pulses as Raman pump, totaling 96 wavelength-shifted Raman pump with 3 µJ energy
- 98 exponentially spaced time delays from 10 fs to 800 ns to sample the photoinduced dynamics of axitinib
- $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ electronic transitions

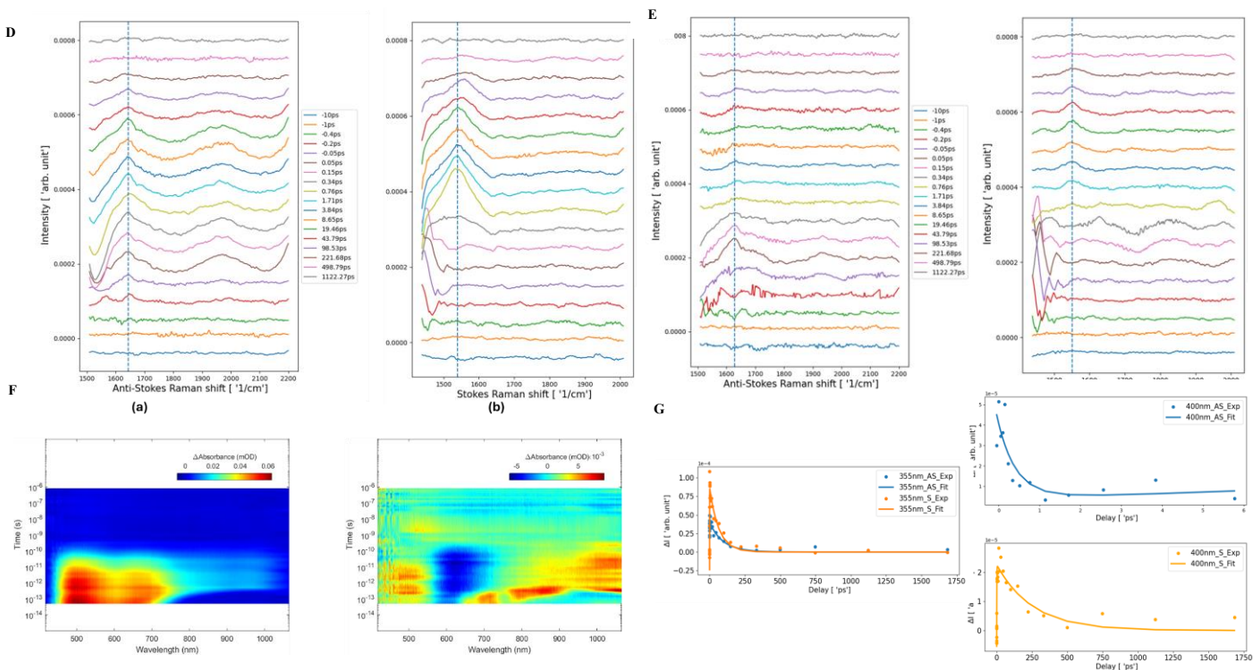


Our setup at ELI Beamlines ERIC in Prague

Result and discussion



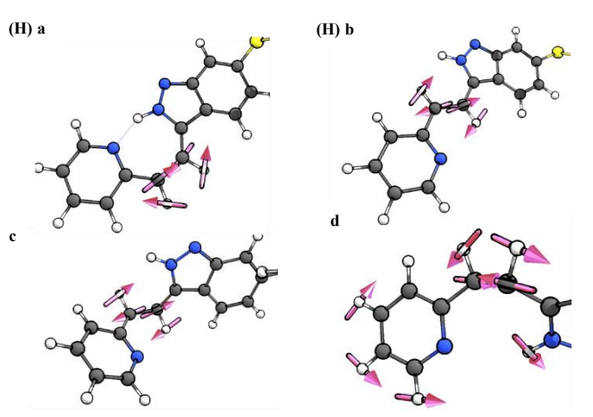


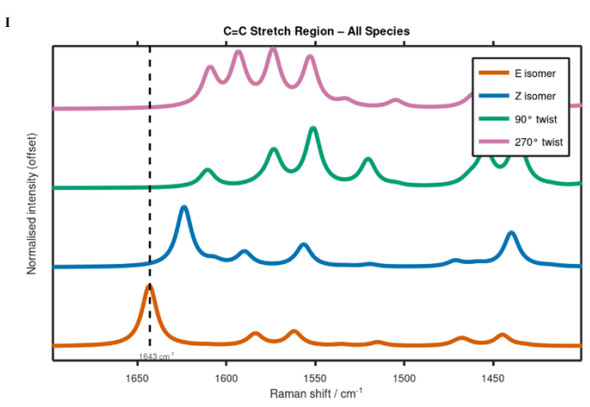


A. Steady-state UV/Vis: The initial sample is dominated by the E **isomer,** broad peak around **340 nm**. To obtain the Z-isomer, the E-isomer was illuminated with an LED centred at **~365 nm** for ~30 minutes. The appearance of the **Z-isomer** population is marked by the emergence of the **peak ~ 385 nm.**

B. The small peak at 1643 cm^{-1} refers to the C=O stretching of the axitinib. The inset in Figure B shows the axitinib C=C heterocycle stretch peak present in Stokes and anti-Stokes Raman spectra of axitinib. The vibrations band at around 1635 cm^{-1} appears to be the most intense band of Axitinib.

C. Lifetime distribution analysis by TA. (i) Normalised average dynamical content (D) as a function of lifetime (τ). Three main components, labeled ① , ②, and ③ were found. (ii) Normalised decay-associated difference spectra (DADS) obtained by integration of the transient spectra in the time range indicated by the shaded areas of panel (i). The position of the main differential absorption bands (in nm) is indicated.





D. Fig. D corresponds to the actinic pump of **355nm**, where the **Stokes shift** has a clear shift (blue shift) in the peak, which is a signature of the **isomerisation**. In anti-Stokes spectra, there is potential broadening of the peak up to **0.76 ps**. There is a strange peak evolving at around **1970 cm^{-1}** until **98 ps**, which cannot be explained

E: Here for 400 actinic pump, in Stokes, there is a change in the Raman peak intensity but no obvious shift

F: (a) 355 nm pump (b) 400 nm pump. Only nanosecond components were detected from preliminary analysis

G: The Z and E states show one intense mode each, corresponding to the ethylene stretch (1637 cm^{-1} and 1657 cm^{-1}, respectively). The Z state lies 1.47 kcal/mol lower in energy than the E state, aided by the hydrogen bond present in this state.

H: The C=C frequency vectors for a) the Z-isomere (1624 cm^{-1}) , b) the E-isomere (1643 cm-1), c) the 270 degree twist (1574 cm-1), and d) the 90 degree twist(1551 cm^{-1}). The values are after scaling the calculated frequencies by 0.977 to match the E-isomer Raman intensity at 1643 cm^{-1}.

I: The calculated Raman spectra of all four states. The two twisted conformations have contributions from the C=C bond and other parts of the molecule. As expected, the weaker bond shifts the frequency lower for these two conformations.

Conclusion

- To summarise, photoexcitation of molecular crystals often induces structural transformations or loss of crystallinity, yet the mechanistic connection between ultrafast molecular dynamics and the resulting lattice response remains poorly understood.
- Here, we combine femtosecond stimulated Raman spectroscopy, powder X-ray diffraction, and single-nanocrystal electron diffraction to demonstrate that the structural evolution of a photoactive molecular crystal is governed by a dominant torsional reaction coordinate.
- In this work, we investigate the ultrafast photophysics of the cis–trans isomerisation of axitinib. E–Z (or cis–trans) photoswitches are the most widely used in reversible photopharmacology.
- These switches undergo light-induced isomerisation between the E (trans) and Z (cis) forms, thereby altering the molecular conformation and, consequently, the biological activity. In recent years, E–Z photoswitches have found increasing application as functional moieties in the design of photopharmacological agents.
- The same vibrational signature observed in both Stokes and anti-Stokes FSRS spectra identifies a preferentially populated torsional mode, while crystallographic analysis reveals that this motion is sterically constrained within the lattice.
- Upon illumination, the crystal exhibits anisotropic structural evolution, including pronounced changes along the b axis and variations in unit-cell angle, indicating a directional lattice response rather than simple thermal disorder.
- Time-dependent PXRD and Q-dependent electron diffraction further reveal non-monotonic evolution from transient structural reorganisation to progressive loss of long-range order.
- Together, these results establish a direct multiscale connection between a specific molecular vibration and the nonequilibrium structural fate of the crystal lattice.

Reference

1) Lórenz-Fonfría, V. A. & Kandori, H. Transformation of Time-Resolved Spectra to Lifetime-Resolved Spectra by Maximum Entropy Inversion of the Laplace Transform. *Applied Spectroscopy* **60**, 407-417, doi:10.1366/000370206776593654 (2016).
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3) Chaudhari, A. S. *et al.* Genetically encoded non-canonical amino acids reveal asynchronous dark reversion of chromophore, backbone, and side-chains in EL222. *Protein Science* **32**, doi:10.1002/pro.4590 (2023).
4) Liu, Y. *et al.* Sub-Millisecond Photoinduced Dynamics of Free and EL222-Bound FMN by Stimulated Raman and Visible Absorption Spectroscopies. *Biomolecules* **13**, doi:10.3390/biom13010161 (2023).