A handle on charge reorganization

Grozema, Ferdinand C.

DOI
10.1038/s41557-022-00987-0

Publication date
2022

Document Version
Final published version

Published in
Nature Chemistry

have previously been described[7], they do not have sufficient stability for optimal delivery by antibodies. Jiménez-Osés, Bernardes and co-workers saw an opportunity to devise a new prodrug strategy that would enable a stable and reversible linkage (Fig. 1). They envisioned using a *para*-aminobenzyl self-immolating spacer where the aniline would, through resonance stabilization, bring about the elimination of the hydroxyl group of a hydroquinone, which would then oxidize to the desired *ortho*-quinone product.

Serendipitously, during the attempted synthesis of the O-benzyl product, the team instead obtained a C-benzyl derivative. Upon generation of the desired aniline intermediate, they observed a 1,6-elimination and simultaneous oxidation to generate the free *ortho*-quinone. Further study of the elimination reaction uncovered a pH dependence for the elimination; slow and incomplete release was observed under basic conditions, however, acidic conditions enabled successful drug release. Importantly, this new linking strategy was confirmed to be both stable in blood and prevent the redox cycling that leads to the off-target toxicity.

Next, the team designed and assembled a β-lapachone-containing ADC for inhibiting the growth of acute myeloid leukaemia cells. They used an antibody found in the clinically approved ADC Mylotarg, to target CD33, a well validated tumour-associated antigen found on the surface of acute myeloid leukaemia cells. The anti-CD33-β-lapachone ADC containing the linker was active in vitro and also inhibited tumour-growth in a murine model of acute myeloid leukaemia at tolerated doses. This is a quite unexpected and interesting result because one significant challenge in the ADC field is the requirement to use drugs with extremely high potencies when in the free drug form to ensure that the administered drug-conjugates are active. This restriction arises from the (often limited) amount of drug that can be delivered intracellularly when target saturation occurs.

Although conjugate potency is dependent on many factors — including drug loading (drug-to-antibody ratio), antigen expression, conjugate internalization and the intracellular retention of the drug (and likely many other factors) — the free drug typically needs to have an IC_{50} in the picomolar range for an ADC to show anti-tumour activity. Yet, despite β-lapachone having a free drug potency of ~1 μM, the resulting conjugate was active. It is surprising that such a low potency payload affords an active ADC, especially considering the low drug loading (drug-to-antibody ratio of 2) and quite low antigen expression for CD33. An intriguing possibility is that β-lapachone can enact cytotoxic effects upon drug release in the lysosome and not rely on escape from this compartment. There are precedents for ADCs, with the site of action being the endosome or lysosome, to potentially have less stringent potency requirements[8,9]. With this in mind, further studies looking at the impact of the mechanism and site of action on free drug potency requirements for ADCs are warranted.

There is a tremendous amount of work required before any ADCs based on this chemistry can be developed into a useful therapeutic, but it is clear that this method is an interesting approach for linking *ortho*-quinones to a protein carrier, and that it will enable the exploration of new ADCs, and further our understanding of how to make potent and safe ADCs. ❐

Thomas Pillow ✉
Genentech, South San Francisco, CA, USA.
✉*e-mail: pillow.thomas@gene.com*

Published online: 1 July 2022
https://doi.org/10.1038/s41557-022-00993-2

References
1. Drago, J. Z., Modi, S. & Chandarlapaty, S. *Nat. Rev. Clin. Oncol.* **18**, 327–344 (2021).
2. Staben, L. R. et al. *Nat. Chem.* **8**, 1112–1119 (2016).
3. Kolakowski, R. V. et al. *Angew. Chem. Int. Ed.* **55**, 7948–7951 (2016).
4. Dunsmore, L. et al. *Nat. Chem.* https://doi.org/10.1038/s41557-022-00964-7 (2022).
5. Chuprakov, S. et al. *Bioconjug. Chem.* **32**, 746–754 (2021).
6. Gong, Q., Hu, J., Wang, P., Li, X. & Zhang, X. *Eur. J. Med. Chem.* **210**, 112962 (2020).
7. Ma, X. et al. *J. Control. Release* **200**, 201–211 (2015).
8. Lehar, S. M. et al. *Nature* **527**, 323–328 (2015).
9. Gadd, A. J. R., Greco, F., Cobb, A. J. A. & Edwards, A. D. *Bioconjug. Chem.* **26**, 1743–1752 (2015).

Competing interests
The author declares no competing interests.

Check for updates

PHOTOREDOX CATALYSIS

A handle on charge reorganization

Photoredox catalysts offer a promising approach to performing reactions with high energetic requirements, however, the influence of solvent and counter ions is not fully understood. Now, a microwave-based technique is shown to give direct insight into their effects on charge reorganization during catalysis.

Ferdinand C. Grozema

The rearrangement of charge is a central aspect in many light-driven processes in biology, chemistry and materials science with the absorption of light often triggering a plethora of processes during which electrons — and often nuclei — reorganize in molecules. Clear examples are found in natural photosynthesis, in which a key step is the formation of a charge-separated state, and in organic solar cells, where the interface between an electron-donating and an accepting material induces the formation of isolated charges. The time-resolved detection of such charge rearrangement is usually indirect, for instance, exploiting changes in optical absorption that characterize the different species that are formed, but the assignment of optical signatures to specific species can be difficult to do in an unambiguous way.

Now, writing in *Nature Chemistry*, Rumbles, Reid and co-workers describe[1] a more direct technique for probing the rearrangement of charge following the absorption of light and use it to characterize the ion-pair reorganization that occurs in photoredox catalysts after photoexcitation. The technique, time-resolved dielectric loss spectroscopy (TRDL, and also called time-resolved microwave conductivity) has

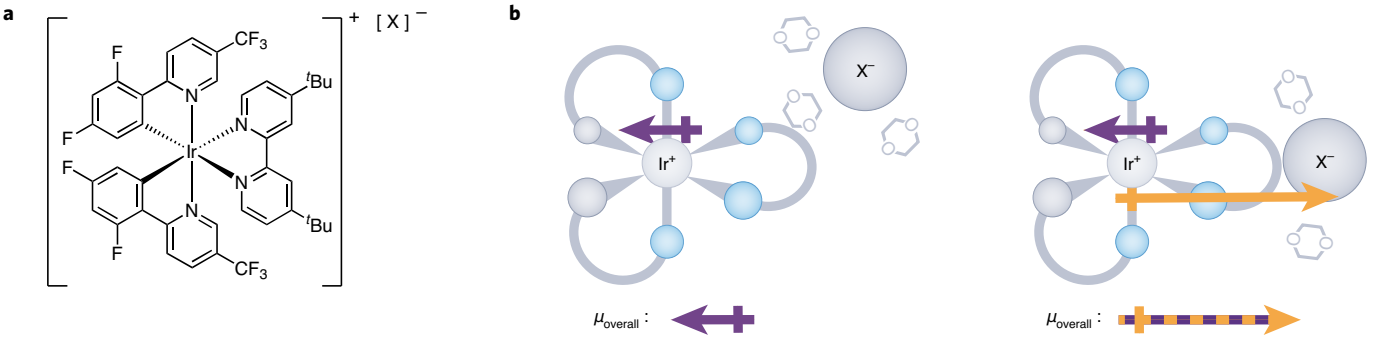

Fig. 1 | Structure of photoredox catalysts. a, Structure of the [Ir[dF(CF$_3$)ppy]$_2$(dtbpy)]X compound, where X$^-$ represents the counter ions BAr$^F{}_4{}^-$ and PF$_6{}^-$. **b**, The proximity of the counter ion strongly affects the overall charge distribution and the catalytic activity. Credit: adapted from ref. [1]., Springer Nature Ltd.

its origin in radiation chemistry in the 1970s where it was used for the time-resolved detection of ionic species made by pulsed irradiation[2]. Among the pioneers of this technique was John Warman at Delft University of Technology who used TRDL in several forms to study the excited-state properties of molecules in solution and charge transport in dielectric liquids and organic materials[2–4].

In the TRDL approach, microwaves are used to probe changes in the dielectric properties of a sample after irradiation with a short pulse from a laser or an electron accelerator[5]. This dielectric response is a complex quantity, consisting of a real and an imaginary part. The real part corresponds to the transient changes in the dielectric constant (or the ability of an electric field to polarize the charge distribution), and the imaginary part describes the dielectric loss. This latter loss component is interesting as it corresponds to the absorption of microwaves and can be interpreted as a conductivity. As a result, TRDL has been used extensively by a limited number of groups to perform time-resolved conductivity measurements, for example, photoconductivity measurements materials for solar cells or determinations of charge carrier mobilities in organic semiconductors[5,6].

Although less common, TRDL can also be used to measure excited-state dipole moments. Dipolar species can absorb microwave power through rotational motion, the same phenomenon used to heat food in a microwave oven, which works through the rotational motion of dipolar water molecules. If the dipole moment of a molecule in solution changes on photoexcitation it can be measured via losses in the transient dielectric signal. Such experiments played a key role in proving that full charge separation can take place over large distances in donor–bridge–acceptor systems[3].

In all of the examples above, it is the dielectric loss (or the imaginary part of the dielectric constant) that determines the response. Changes in the real dielectric constant are related to the ease with which a charge distribution can be polarized, for instance, when measuring excited-state polarizabilities of conjugated molecules to gain insight into the nature and degree of delocalization of excited states. Change in the real dielectric constant are also observed in the case of a very rapid intramolecular rearrangement of the dipole moment.

Microwave-based dielectric loss spectroscopy methods therefore offer a direct handle on charge distribution in molecular systems in solution. Rumbles and co-workers use the TRDL approach to unravel some intriguing aspects of photoredox catalysts[1]. Such catalysts can drive reactions with high kinetic or thermodynamic barriers using the absorption of photons. Common photoredox catalysts consist of a d_6-metal centre — like the iridium complex interrogated by Rumbles, Reid and colleagues (Fig. 1a) — surrounded by multiple ligands. When they are photoexcited, metal-to-ligand charge-transfer states are generated that exhibit substantial changes in charge distribution, and hence a substantial change in dipole moment compared to the ground state. The team's TRDL data show that this charge rearrangement is closely coupled to the surroundings of the complex, and counter ions that form a closely bound ion pair with the metal complex are shown to substantially affect the charge distribution in the excited state. The size and polarity of the counter ion were also seen to strongly affect the reactivity of the photocatalyst, pointing to the effects of ion pairing.

It is almost impossible to quantify such ion-pairing effects using optical measurements, but Rumbles, Reid and colleagues have used a revitalized form of TRDL to gain direct insight into the charge distribution in the ground and excited states of Ir complexes with different counter ions. Their ground-state measurements showed that small negative counterions (PF$_6{}^-$ in this case) form tightly bound ion pairs that behave as a permanent dipole moment, generating a dielectric loss signal. The team were also able to derive the overall dipole moment using a new quantitative modelling approach. When they replaced the small counter ion by a much larger one (BAr$^F{}_4{}^-$) they saw the Ir complexes behave as individual ions in solution, rather than forming tightly bound ion pairs, and hence the dipole moment of the ground-state complex itself determined the dielectric loss signal (Fig. 1b).

In the excited state the results become even more intriguing. From measuring the changes in the real and imaginary dielectric constant, a detailed picture emerges where the ion pairing not only affects the magnitude of the dipole moment, but also directs the nature of the excited state itself through the electric field that it exerts on the Ir complex. The effects of the counter ions on the nature of the excited state and the specific polarization that their presence induce have direct consequences for the catalytic activity, resulting in up to a fourfold increase in reaction rate when ion-pairing is removed. These results give important new insights into the effects of pairing with counter ions and solvent polarity on the catalytic activity of this whole class of photoredox catalysts.

The work of Rumbles, Reid and colleagues may extend further than just the specifics of the photoredox systems studied here. They show that reviving a somewhat eccentric form of dielectric loss spectroscopy and combining it with modern numerical modelling approaches can result in a unique method by which to shine a direct light on excited-state charge distribution in molecular systems. This will certainly lead to many more insights into other photoinduced

processes that are almost impossible to obtain by other techniques. ❐

Ferdinand C. Grozema
Department of Chemical Engineering, Delft University of Technology, Delft, The Netherlands.
✉*e-mail: F.C.Grozema@tudelft.nl*

Published online: 1 July 2022
https://doi.org/10.1038/s41557-022-00987-0

References
1. Earley, J. D. et al. *Nat. Chem.* https://doi.org/10.1038/s41557-022-00911-6 (2022).
2. Warman, J. M., Dehaas, M. P. & Hummel, A. *Chem. Phys. Lett.* **22**, 480–483 (1973).
3. Warman, J. M. et al. *Nature* **320**, 615–616 (1986).
4. Schouten, P. G., Warman, J. M., Dehaas, M. P., Fox, M. A. & Pan, H. L. *Nature* **353**, 736–737 (1991).
5. Savenije, T. J., Ferguson, A. J., Kopidakis, N. & Rumbles, G. *J. Phys. Chem. C* **117**, 24085–24103 (2013).
6. Warman, J. M. et al. *Chem. Mater.* **16**, 4600–4609 (2004).

Competing interests
The author declares no competing interests.

ULTRAFAST SPECTROSCOPY

Plot twist in the iron spin saga

Over the past 25 years, the photo-induced spin-crossover behaviour of Fe(II) complexes has puzzled scientists. Now, a symmetry-breaking twisting mode has been observed during the relaxation of such a complex. Controlling its configuration using enantiopure counterions has also been shown to slow down the relaxation.

J. Olof Johansson

Switching and reading the spin state of molecules and nanoparticles on ultrafast timescales is important for developing next-generation information storage and quantum technologies. Spin-crossover compounds featuring iron, such as $[Fe^{II}(bpy)_3]^{2+}$ (bpy = 2,2′-bipyridine), have been studied for decades, and it is well-known that a fast change in spin state from $S = 0$ (low spin) to $S = 2$ (high spin) can take place in just 100 fs after optically exciting the metal-to-ligand charge-transfer band[1]. At the time of its discovery, this finding was surprising because of the need to conserve spin angular momentum after absorbing a photon, and the known typical (much longer) timescale for singlet-to-triplet conversion in organic molecules. The charge-transfer state decays into the high-spin metal-centred state concurrently with the expansion of the Fe–N bonds via a breathing mode; however, other non-totally-symmetric modes are also involved in the dynamics[2] and some questions about their nature and role remain open.

As is the case for most chemical physics, the field moves forward when new techniques are introduced. For example, X-ray free-electron lasers have provided great insights into Fe(II) spin-crossover[3,4]. Now, writing in *Nature Chemistry*, Oppermann and colleagues have described the use of time-resolved circular dichroism (TRCD) in the ultraviolet spectral region to interrogate the spin-state dynamics of an iron complex[5]. They identify torsional twisting modes involved in the relaxation of its high-spin state back to its low-spin ground state and show how such relaxation can be slowed through control of its configuration.

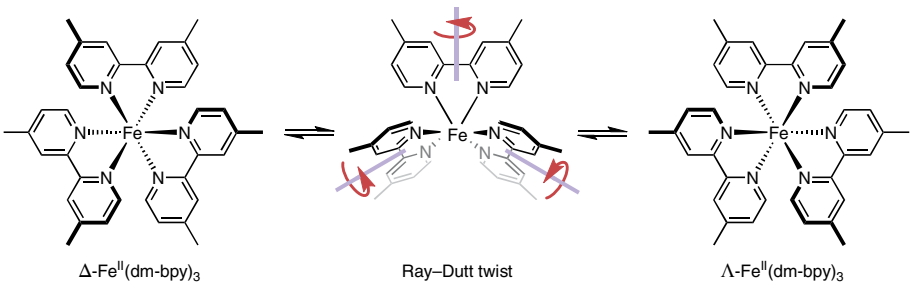

Fig. 1 | The Ray–Dutt twist involved in the racemization of $[Fe^{II}(dm\text{-}bpy)_3]^{2+}$ (dm-bpy = 4,4′-dimethyl-2,2′-bipyridine). The twist plays an important role in the decay of the high-spin state back to the low-spin ground state. Δ and Λ are the two enantiomers.

Circular dichroism (CD) arises because of a difference between absorption of left- and right-handed circularly polarized light by a chiral chromophore. In a time-resolved measurement, CD spectroscopy has the potential to give information on nuclear rearrangements in the excited state because of its structural sensitivity. However, TRCD is a challenging technique because of weak signals: the difference in absorption between left- and right-handed circular light of a typical chiral molecule is on the order of 0.1%, and the time-resolved signal is around 1% of this signal! Furthermore, most optical components will introduce some distortion to the spectrum. Recently, Oppermann and colleagues presented a femtosecond, broadband TRCD spectrometer, demonstrating that they had overcome these challenges and achieved high sensitivity[6]. They have now put this set up to work studying $[Fe^{II}(dm\text{-}bpy)_3]^{2+}$ (dm-bpy = 4,4′-dimethyl-2,2′-bipyridine) (Fig. 1).

The team of scientists used diastereomeric interactions between their iron complex and chiral phosphate counterions (TRISPHAT; $P(O_2C_6Cl_4)_3^-$), to generate an enantiopure sample, which was key to enabling their measurements. Initially, using transient absorption (TA) spectroscopy, the team could immediately observe that the lifetime of the high-spin state was four times longer for the enantiopure solution compared to the racemic solution. The team also carried out complementary transient absorption anisotropy (TAA) experiments, which are transient absorption measurements as a function of the angle between the pump and the probe polarization directions. This technique can give information on the