INVESTIGATION OF NONLINEAR OPTICAL PROPERTIES OF OPSINS: TOWARDS OPSIN PROBES FOR CELL MEMBRANE VOLTAGE

Y. De Coene [(1)], S. Van Cleuvenbergen [(3)], C. Van Den Haute [(2)], V. Baekelandt [(2)], K. Thevissen [(4)], Thierry Verbiest [(3)], K. Clays [(3)], C. Bartic [(1)]

(1) Laboratory of Soft Matter and Biophysics, Department of Physics KU Leuven, Celestijnenlaan 200D, B-3001 Leuven, Belgium

(2) Research Group for Neurobiology and Gene therapy, Department of neurosciences, KU Leuven, Kapucijnenvoer 33I, 3000 Leuven, Belgium

(3) Molecular Imaging and Photonics, Department of Chemistry, KU Leuven, Celestijnenlaan 200D, B-3001 Leuven, Belgium

(4) Centre of Microbial and Plant Genetics Kasteelpark Arenberg 20 - box 2460 3001 Leuven, Belgium

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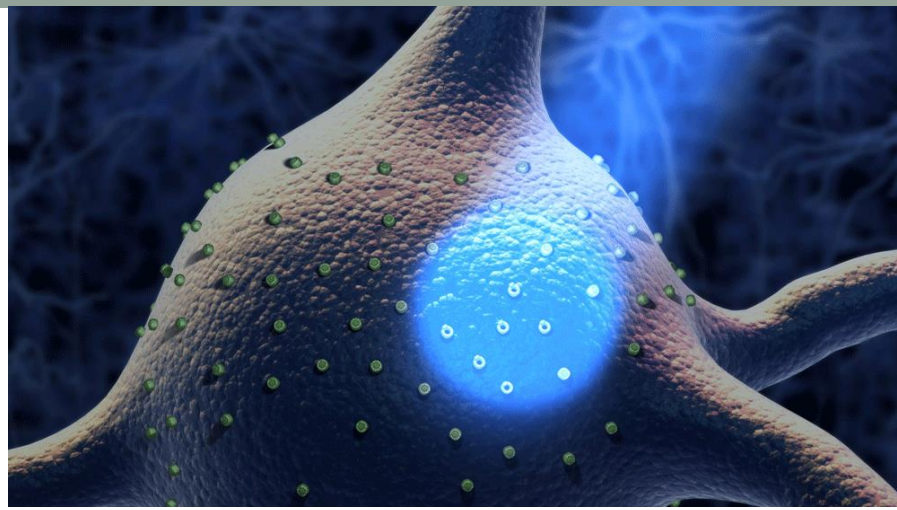
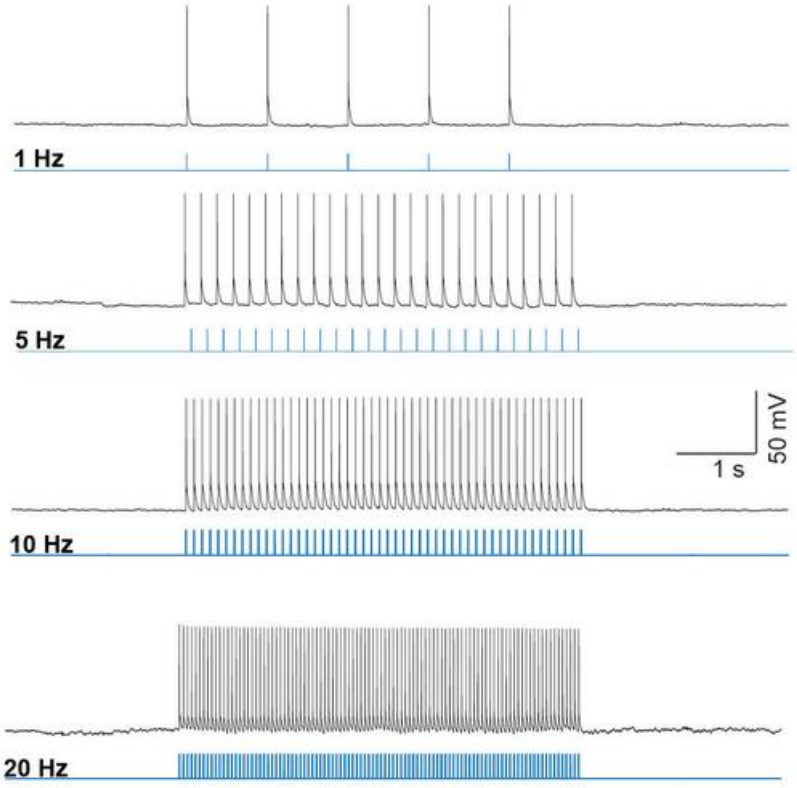
Introduction

- Need for stimulation and recording of neural activity at the same time
- Preferably at single cell level
  - Unravel interconnectivity
- Fast enough to keep up with neuronal firing
  - Millisecond time resolution

Optogenetics

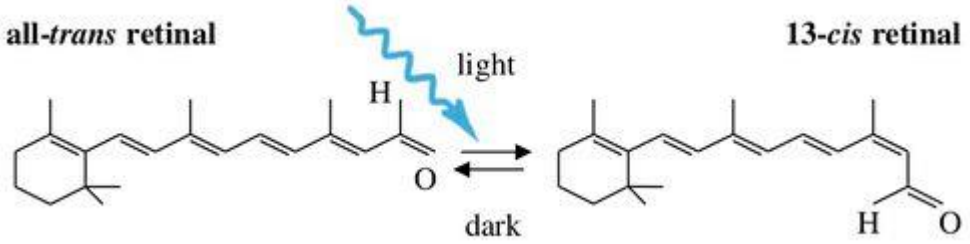
- Light sensitive ion channels
- Genetic techniques
- Temporal resolution
  - milliseconds
- Manipulation
  - Single photon optogenetics
- Read-out
  - voltage sensitive fluorescent probes

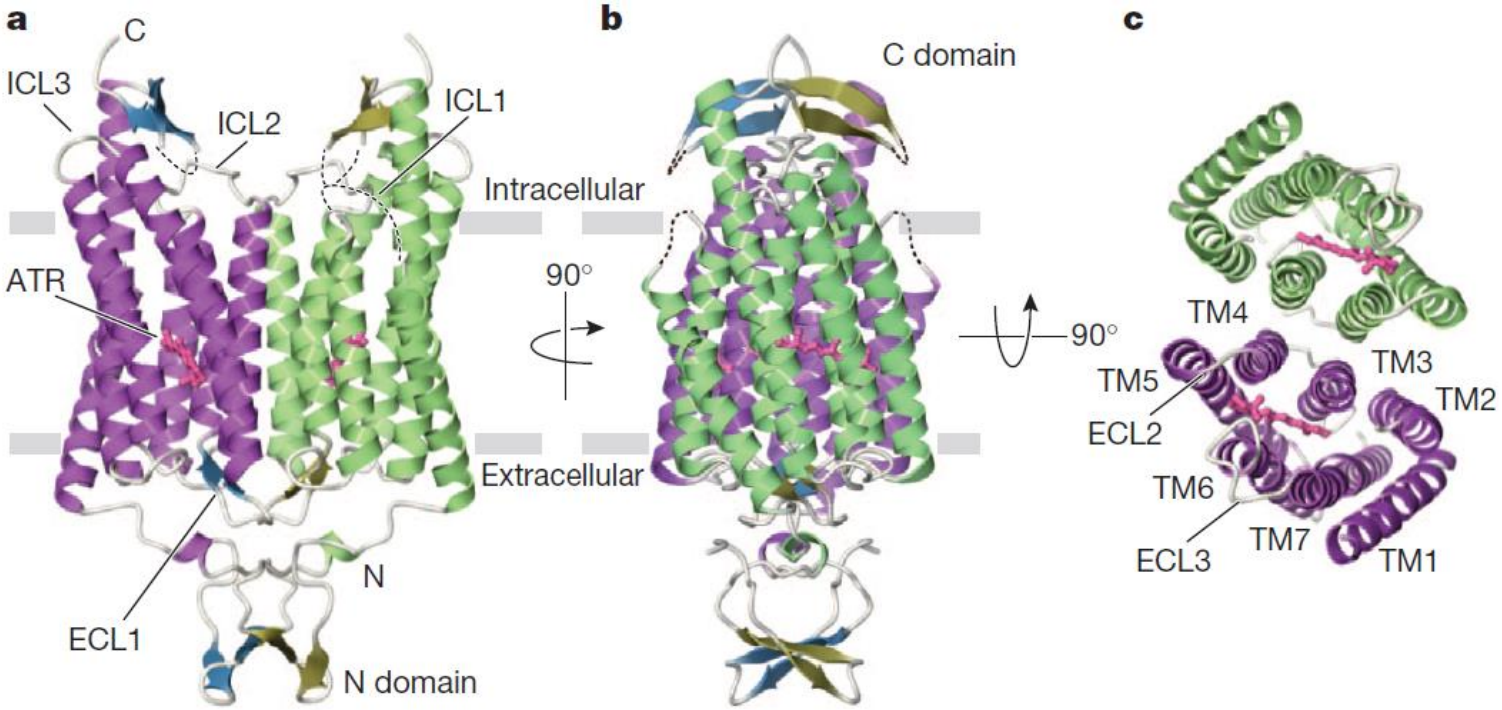




(Herrera et al., Nature neuroscience, 2016)

C1C2





Crystal structure of C1C2. (Kato et al., Nature, 2012)

Towards nonlinear optogenetics

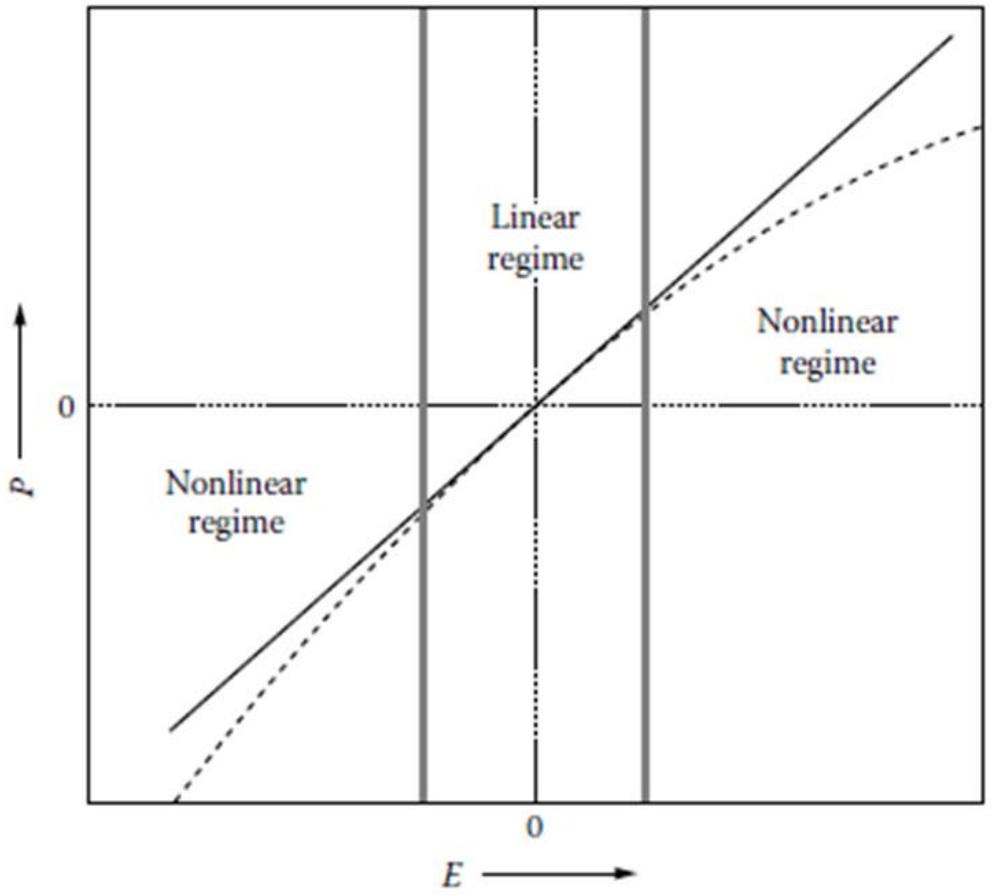
- One probe for activity control AND voltage sensing
- Manipulation of neuronal activity by **two-photon absorption**
  - Higher spatial resolution (subcellular)
  - Higher tissue penetration (up to 800µm)
- Voltage readout using **second harmonic generation**
  - Retinal chromophore

Goal

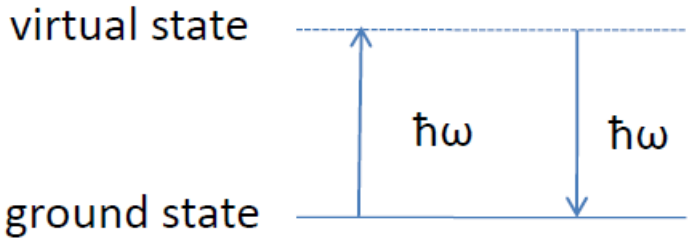
- Recombinantly express and purify channelrhodopsin mutants
- Investigate the first hyperpolarizability β
  - Indicator for SHG probe efficiency
- Compare it to bacteriorhodopsin
  - Only light sensitive protein used in nonlinear optics

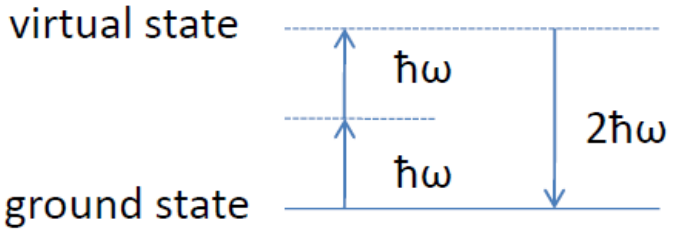
What is ‘β’?

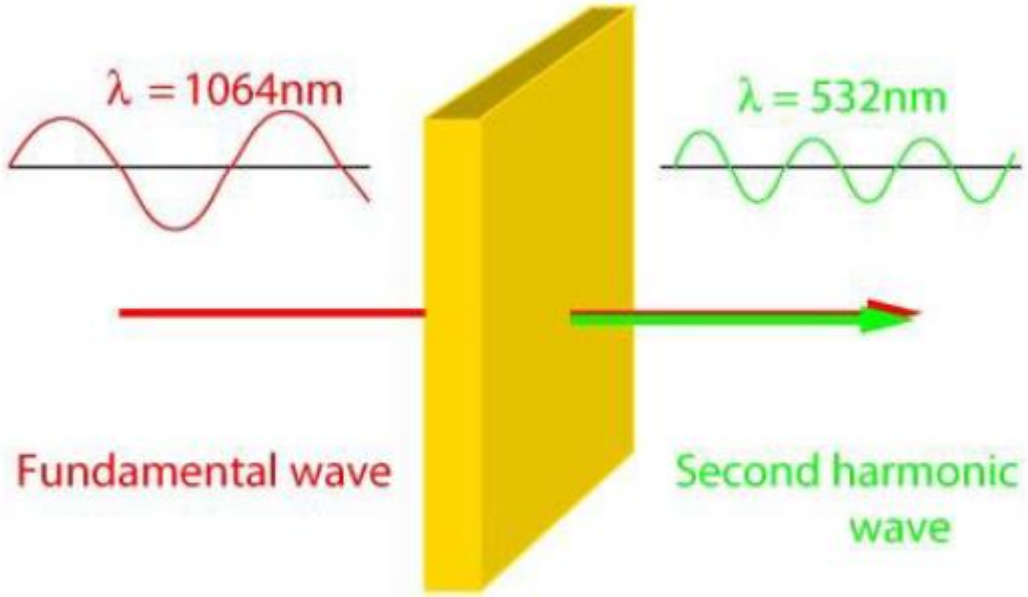
$$\mu_{ind} = \mu^{(1)} + \mu^{(2)} + \mu^{(3)} + \ldots = \alpha E + \beta EE + \gamma EEE + \ldots$$



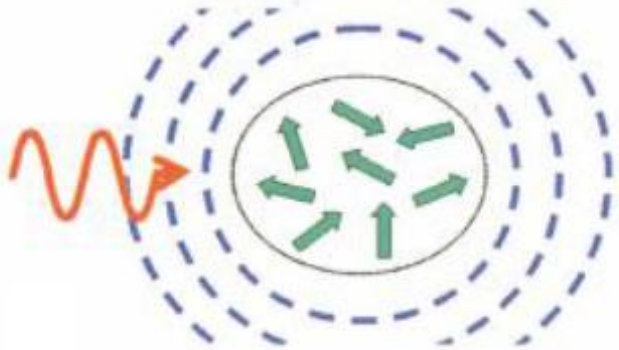
Second harmonic generation

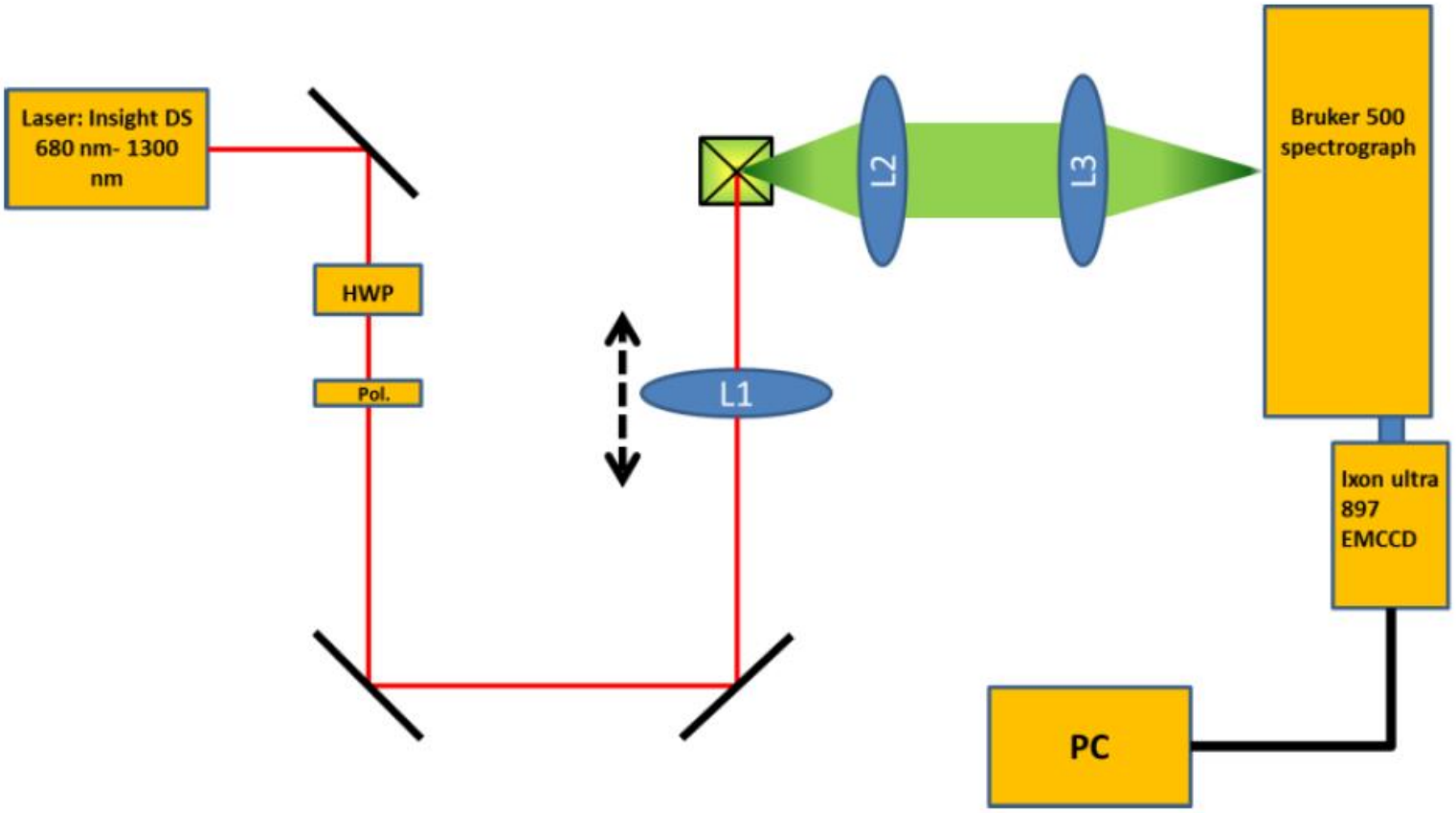






Measuring β?

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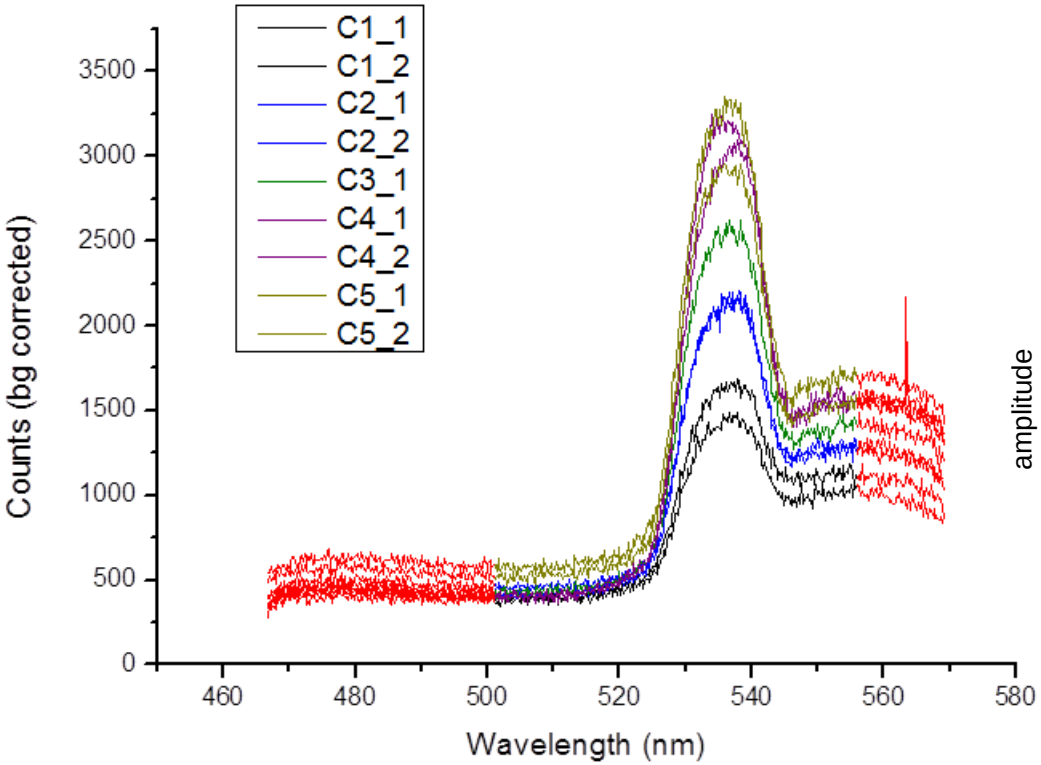
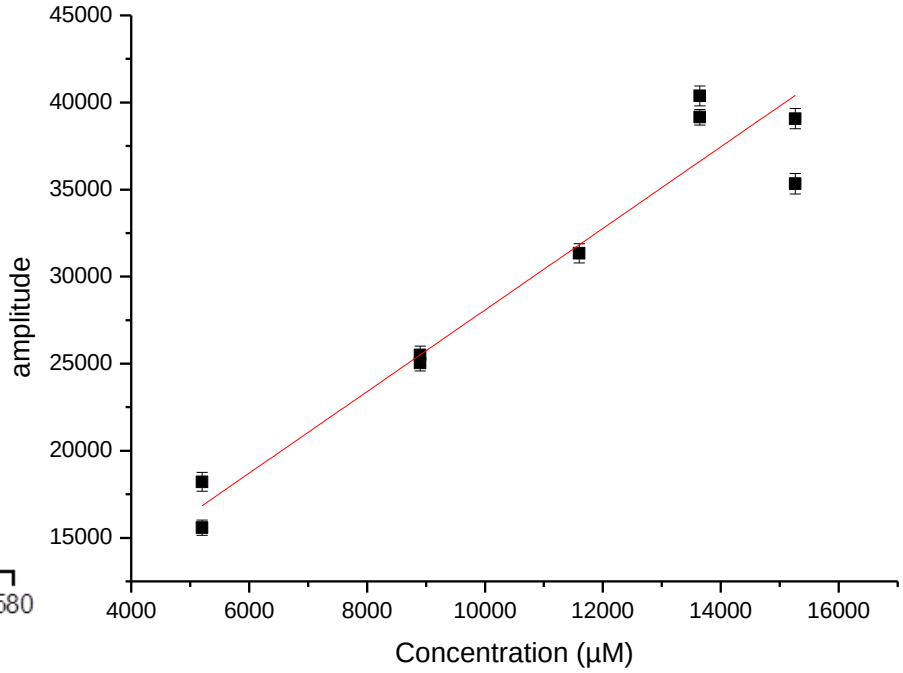
HWP
Pol.
L1
L2
L3
Bruker 500 spectrograph
Ixon ultra 897 EMCCD
PC





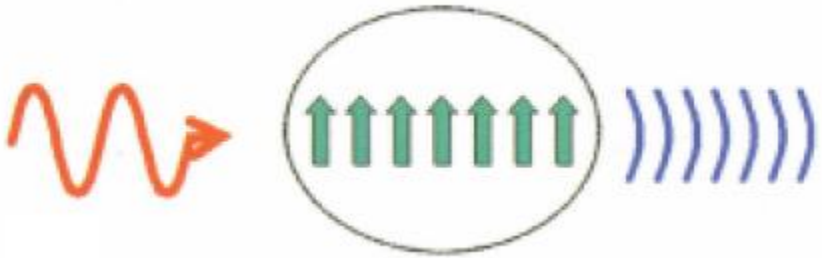
$$I^{2\omega} = \frac{16\pi^5}{c\lambda^4 r^2}.N.f(\omega)^4.f(2\omega)^2.\langle\beta^2_{HRS}\rangle.I(\omega)^2$$

(Clays et al., *Adv. Chem. Phys., 1994*)

$$I^{2\omega} = g.\langle\beta^2_{HRS,X}\rangle.I(\omega)^2$$

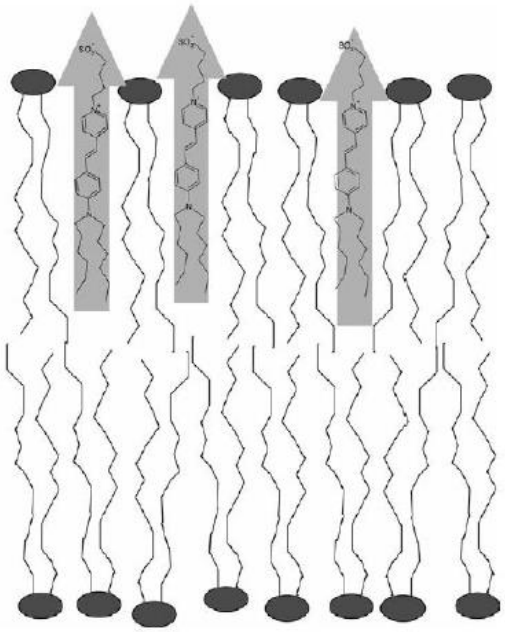
$$I^{2\omega} = g.\langle\beta^2_{HRS,REFERENCE}\rangle.I(\omega)^2$$

Relation to SHG



Correlated chromophores

= individual chromophores with specific and time invariant phase relations with respect to one another



$$I(2\omega) \propto N^2 \beta^2$$

Experimental β values at 1064nm

	$\beta_{hrs,1064nm}$ ($10^{-30} esu$)	
FM4-64	160 ± 30	Commercially available SHG probe
ATR	130 ± 30	Chromophore
C1C2	600 ± 150	Channelrhodopsin mutants. Purified from p.pastoris
ChRH134R	530 ± 130	
bR	**1100 ± 150**	Only characterized opsin

Conclusion and outlook

- Seem to be good candidates for SHG probes
- Already proven in optogenetics
- Test their voltage sensitivity in lipid vesicles
- First step towards all optical control

Thank you for your attention!