

**Jan Steyaert, PhD**

**Full Professor & Francqui Research Professor**, Faculty of Sciences and Bioengineering Sciences, VUB

**Director**, Department of Structural Biology Brussels, VUB

**Acting Director**, Structural Biology Research Center, VIB

**Founder & CSO**, ConFotherapeutics NV, [www.ConFotherapeutics.com](http://www.ConFotherapeutics.com)



## Expertise

Last years, the Steyaert lab pioneered the use of nanobodies for chaperone-assisted X-ray crystallography ([www.steyaertlab.eu](http://www.steyaertlab.eu)), aiming at the highest hanging fruits of structural biology including membrane proteins, amyloidogenic proteins, and now also (transient) multiprotein complexes. The elucidation of the first GPCR structures in the agonist-bound active state demonstrate the power of Nanobodies to stabilize G protein coupled receptor conformational states including transmembrane signalling complexes. Recent work focusses on exploiting the conformational complexity of therapeutic targets for Nanobody-enabled drug discovery.

Apart of his research activities, Jan Steyaert is also a serial entrepreneur. He is a co-founder of Ablynx (now a Sanofi company) and Biotals and more recently he founded ConfoTherapeutics, three successful biotech spin-offs that valorize a unique class of single domain antibodies (Nanobodies) derived from camelids.

## Research output

### **Development, characterization and engineering of anti-LexA nanobodies as suppressors of the bacterial SOS response**

Vascon, F., Maso, L., Chinellato, M., Bouchiba, Y., Campagnaro, E., De Felice, S., Goormaghtigh, F., Bellio, P., Cioci, G., Angelini, A., Celenza, G., Barbe, S., Van Melderen, L., Steyaert, J., Pardon, E., Tondi, D. & Cendron, L., Jul 2022, In: FEBS Open Bio. 12, p. 24-24

### **Structural basis of sodium-dependent bile salt uptake into the liver**

Goutam, K., Ielasi, F. S., Pardon, E., Steyaert, J. & Reyes, N., 30 Jun 2022, In: Nature. 606, 7916, p. 1015-1020 6 p.

### **Stabilization of Meta-I Rhodopsin Conformation by a Nanobody**

Salom, D., Wu, A., Sander, C. L., Pardon, E., Steyaert, J., Kiser, P. D. & Palczewski, K., 1 May 2022, In: The FASEB Journal. 36, S1

### **Snapshots of actin and tubulin folding inside the TRiC chaperonin**

Kelly, J. J., Tranter, D., Pardon, E., Chi, G., Kramer, H., Happonen, L., Knee, K. M., Janz, J. M., Steyaert, J., Bulawa, C., Paavilainen, V. O., Huiskonen, J. T. & Yue, W. W., May 2022, In: Nature Structural & Molecular Biology. 29, 5, p. 420-429 10 p.

### **Conformational transitions and ligand-binding to a muscle-type nicotinic acetylcholine receptor**

Zarkadas, E., Pebay-Peyroula, E., Thompson, M. J., Schoehn, G., Uchański, T., Steyaert, J., Chipot, C., Dehez, F., Baenziger, J. E. & Nury, H., 20 Apr 2022, In: Neuron. 110, 8, p. 1358-1370.e5 13 p.

### **Structure, substrate recognition and initiation of hyaluronan synthase**

Maloney, F. P., Kuklewicz, J., Corey, R. A., Bi, Y., Ho, R., Mateusiak, L., Pardon, E., Steyaert, J., Stansfeld, P. J. & Zimmer, J., 7 Apr 2022, In: Nature. 604, 7904, p. 195-201 7 p.

### **Nanobodies as allosteric modulators of Parkinson's disease-associated LRRK2**

Singh, R. K., Soliman, A., Guaitoli, G., Störmer, E., von Zweydford, F., Dal Maso, T., Oun, A., Van Rillaer, L., Schmidt, S. H., Chatterjee, D., David, J. A., Pardon, E., Schwartz, T. U., Knapp, S., Kennedy, E. J., Steyaert, J., Herberg, F. W., Kortholt, A., Gloeckner, C. J. & Versées, W., 1 Mar 2022, In: Proceedings of the National Academy of Sciences of the United States of America. 119, 9, p. 1-12 12 p., e2112712119.

### **Mapping inhibitory sites on the RNA polymerase of the 1918 pandemic influenza virus using nanobodies**

Keown, J. R., Zhu, Z., Carrique, L., Fan, H., Walker, A. P., Serna Martin, I., Pardon, E., Steyaert, J., Fodor, E. & Grimes, J. M., 11 Jan 2022, In: Nature Communications. 13, 1, 11 p., 251.

### **Cryo-EM Structure of an Atypical Proton-Coupled Peptide Transporter: Di- and Tripeptide Permease C**

Killer, M., Finocchio, G., Mertens, H. D. T., Svergun, D. I., Pardon, E., Steyaert, J. & Löw, C., 2022, In: *Frontiers in molecular biosciences*. 9, p. 1-15 15 p., 917725.

### **Structure, mechanism, and inhibition of Hedgehog acyltransferase**

Coupland, C. E., Andrei, S. A., Ansell, T. B., Carrique, L., Kumar, P., Sefer, L., Schwab, R. A., Byrne, E. F. X., Pardon, E., Steyaert, J., Magee, A. I., Lanyon-Hogg, T., Sansom, M. S. P., Tate, E. W. & Siebold, C., 9 Dec 2021, In: *Molecular Cell*. 81, 24, p. 5025-5038 14 p., 5038.e10.

### **HDX-MS-optimized approach to characterize nanobodies as tools for biochemical and structural studies of class IB phosphoinositide 3-kinases**

Rathinaswamy, M. K., Fleming, K. D., Dalwadi, U., Pardon, E., Harris, N. J., Yip, C. K., Steyaert, J. & Burke, J. E., 2 Dec 2021, In: *Structure*. 29, 12, p. 1371-1381.e6 28 p.

### **Development of a universal nanobody-binding Fab module for fiducial-assisted cryo-EM studies of membrane proteins**

Bloch, J. S., Mukherjee, S., Kowal, J., Filippova, E. V., Niederer, M., Pardon, E., Steyaert, J., Kossiakoff, A. A. & Locher, K. P., 23 Nov 2021, In: *Proceedings of the National Academy of Sciences of the United States of America*. 118, 47, e2115435118.

### **Nanobody-aided crystallization of the transcription regulator PaaR2 from Escherichia coli O157:H7**

De Bruyn, P., Prolic Kalinsek, M., Vandervelde, A., Malfait, M., Sterckx, Y., Sobott, F., Hadzi, S., Pardon, E., Steyaert, J. & Loris, R., 1 Oct 2021, In: *Acta Crystallographica Section F - Structural Biology Communications*. 77, 10, p. 374-384 11 p., 11.

### **Structure of autoinhibited Akt1 reveals mechanism of PIP3-mediated activation**

Truebestein, L., Hornegger, H., Anrather, D., Hartl, M., Fleming, K. D., Stariha, J. T. B., Pardon, E., Steyaert, J., Burke, J. E. & Leonard, T. A., 17 Aug 2021, In: *Proceedings of the National Academy of Sciences of the United States of America*. 118, 33, 11 p., e2101496118.

### **A topological switch in CFTR modulates channel activity and sensitivity to unfolding**

Scholl, D., Sigoillot, M., Overtus, M., Martinez, R. C., Martens, C., Wang, Y., Pardon, E., Laeremans, T., Garcia-Pino, A., Steyaert, J., Sheppard, D. N., Hendrix, J. & Govaerts, C., 2 Aug 2021, In: *Nature Chemical Biology*. 17, 9, p. 989-997 9 p.

### **Nano-scale resolution of native retinal rod disk membranes reveals differences in lipid composition**

Sander, C. L., Sears, A. E., Pinto, A. F. M., Choi, E. H., Kahremany, S., Gao, F., Salom, D., Jin, H., Pardon, E., Suh, S., Dong, Z., Steyaert, J., Saghatelian, A., Skowronska-Krawczyk, D., Kiser, P. D. & Palczewski, K., 2 Aug 2021, In: *The Journal of Cell Biology*. 220, 8, e20210106.

### **Structure of the phosphoinositide 3-kinase (PI3K) p110 $\gamma$ -p101 complex reveals molecular mechanism of GPCR activation**

Rathinaswamy, M. K., Dalwadi, U., Fleming, K. D., Adams, C., Stariha, J. T. B., Pardon, E., Baek, M., Vadas, O., DiMaio, F., Steyaert, J., Hansen, S. D., Yip, C. K. & Burke, J. E., Aug 2021, In: *Science Advances*. 7, 35, eabj4282.

### **New insights into molecular mechanism of signaling by endocrine FGFs**

Kuzina, E., Ung, P., Mohanty, J., Tome, F., Choi, J., Pardon, E., Steyaert, J., Lax, I., Schlessinger, A. S., Schlessinger, J. & Lee, S., Jul 2021, In: *FEBS Open Bio*. 11, p. 217-217

### **Nanobody exchange chromatography**

Steyaert, J., Pardon, E., Wohlkönig, A., Zögg, T., Kalichuk, V., De Keyser, P. & Fischer, B., 24 Jun 2021, Patent No. WO2021123360A1, 18 Dec 2020, Priority date 20 Dec 2019, Priority No. EP19219043

### **Structure of Nanobody Nb23**

Percipalle, M., Hunashal, Y., Steyaert, J., Fogolari, F. & Esposito, G., 11 Jun 2021, In: *Molecules*. 26, 12, 21 p., 3567.

#### **Positive allosteric modulators of the calcium-sensing receptor**

Steyaert, J., Pardon, E., Wohlkönig, A., Zögg, T., Mos, I., BRÄUNER-OSBORNE, H. & MOSOLFF MATHIESEN, J., 3 Jun 2021, Patent No. WO2021105438A1, 27 Nov 2020, Priority date 27 Nov 2019, Priority No. EP19211709

#### **Dissecting lipid contents in the distinct regions of native retinal rod disk membranes**

Sander, C., Sears, A., Pinto, A., Choi, E., Kahremany, S., Pry, H., Pardon, E., Suh, S., Dong, Z., Steyaert, J., Saghatelian, A., Skowronska-Krawczyk, D., Kiser, P. & Palczewski, K., Jun 2021, In: Investigative Ophthalmology & Visual Science. 62, 8

#### **Nanodisc-specific antigen-binding chimeric proteins**

Steyaert, J., Uchanski, T., Aricescu, A. R. & Masiulis, S., 29 Apr 2021, Patent No. WO2021078786A1, 21 Oct 2020, Priority date 21 Oct 2019, Priority No. EP19204412

#### **In Vivo and In Vitro Characterizations of Nanobodies Raised Against the Melibiose Permease melB of Salmonella Typhimurium**

Katsube, S., Willibal, K., Tikhonova, E. B., Parameswaran, H., Vemulapally, S., Pardon, E., Kaback, H. R., Steyaert, J. & Guan, L., 12 Feb 2021, In: Biophysical journal. 120, 3, p. 74A-74A

#### **Constructing and purifying megabodies starting from individual nanobody sequences**

Uchański, T., Fischer, B., Kalichuk, V., Wohlkönig, A., Zögg, T., Pardon, E. & Steyaert, J., 7 Jan 2021, 11 p.

#### **Megabodies expand the nanobody toolkit for protein structure determination by single-particle cryo-EM**

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#### **Fusion protein with a toxin and scaffold protein**

Steyaert, J., Pardon, E. & Vranken, W., 2021, Patent No. EP3898658

#### **Fusion proteins comprising a cytokine and scaffold protein**

Steyaert, J., Pardon, E., Wohlkönig, A., Kalichuk, V., Vranken, W., Uchanski, T., Chevigné, A. & Szpakowska, M., 2021, Patent No. EP3898664

#### **Protein binding domains stabilizing functional conformational states of gpcrs and uses thereof**

Steyaert, J., Pardon, E., Rasmussen, S. G. F., Fung, J. J., Kobilka, B. K. & Laeremans, T., 2021, Patent No. EP3557255

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#### **A structure of substrate-bound Synaptojanin1 provides new insights in its mechanism and the effect of disease mutations**

Paesmans, J., Martin, E., Deckers, B., Berghmans, M., Sethi, R., Loeys, Y., Pardon, E., Steyaert, J., Verstreken, P., Galicia, C. & Versées, W., 22 Dec 2020, In: eLife. 9, p. 1-27 27 p., e64922.

#### **In vitro reconstitution of dynamically interacting integral membrane subunits of energy-coupling factor transporters**

Setyawati, I., Stanek, W. K., Majsnerowska, M., Swier, L. J. Y. M., Pardon, E., Steyaert, J., Guskov, A. & Slotboom, D. J., 22 Dec 2020, In: eLife. 9, p. 1-21 21 p., e64389.

### **Discussions on the quality of antibodies are no reason to ban animal immunization**

Custers, R. & Steyaert, J., 3 Dec 2020, In: EMBO Reports. 21, 12, e51761.

### **Modular transient nanoclustering of activated $\beta$ 2-adrenergic receptors revealed by single-molecule tracking of conformation-specific nanobodies**

Gormal, R. S., Padmanabhan, P., Kasula, R., Bademosi, A. T., Coakley, S., Giacomotto, J., Blum, A., Joensuu, M., Wallis, T. P., Lo, H. P., Budnar, S., Rae, J., Ferguson, C., Bastiani, M., Thomas, W. G., Pardon, E., Steyaert, J., Yap, A. S., Goodhill, G. J., Hilliard, M. A. & 2 others, Parton, R. G. & Meunier, F. A., 1 Dec 2020, In: Proceedings of the National Academy of Sciences of the United States of America. 117, 48, p. 30476-30487 12 p.

### **Nanobody-enabled monitoring of kappa opioid receptor states**

Che, T., English, J., Krumm, B. E., Kim, K., Pardon, E., Olsen, R. H. J., Wang, S., Zhang, S., Diberto, J. F., Sciaky, N., Carroll, F. I., Steyaert, J., Wacker, D. & Roth, B. L., 1 Dec 2020, In: Nature Communications. 11, 1, p. 1145 12 p., 1145.

### **The G-Protein Rab5A Activates VPS34 Complex II, a Class III PI3K, by a Dual Regulatory Mechanism**

Buckles, T. C., Ohashi, Y., Tremel, S., McLaughlin, S. H., Pardon, E., Steyaert, J., Gordon, M. T., Williams, R. L. & Falke, J. J., 1 Dec 2020, In: Biophysical Journal. 119, 11, p. 2205-2218 13 p.

### **Diversity in kinetics correlated with structure in nano body-stabilized LacY**

Kumar, H., Finer-Moore, J., Smirnova, I., Kasho, V., Pardon, E., Steyaert, J., Kaback, H. R. & Stroud, R. M., May 2020, In: PLOS ONE. 15, 5, 14 p., e0232846.

### **Methods to select agents that stabilize protein complexes**

Steyaert, J., Wohlkönig, A. & Triest, S., 1 Apr 2020, Patent No. EP3194976B, 17 Jul 2015, Priority date 22 Jul 2014, Priority No. EP14178012.2

### **Allosteric modulation of the GTPase activity of a bacterial LRRK2 homolog by conformation-specific Nanobodies**

Leemans, M., Galicia, C., Deyaert, E., Daems, E., Krause, L., Paesmans, J., Pardon, E., Steyaert, J., Kortholt, A., Sobott, F., Klostermeier, D. & Versees, W., Apr 2020, In: Biochemical Journal. 477, 7, p. 1203-1218 16 p.

### **Structure of the G protein chaperone and guanine nucleotide exchange factor Ric-8A bound to Gai1**

McClelland, L. J., Zhang, K., Mou, T-C., Johnston, J., Yates-Hansen, C., Li, S., Thomas, C. J., Doukov, T. I., Triest, S., Wohlkonig, A., Tall, G. G., Steyaert, J., Chiu, W. & Sprang, S. R., 26 Feb 2020, In: Nature Communications. 11, 1, 1077.

### **A Lipid Recognition Site at a Transmembrane Helix Kink Shapes the Agonist Response of a Pentameric Ligand-gated Ion Channel**

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### **Ric8A-G alpha, a Complex Structure of a Guanine Nucleotide Exchange Factor**

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### **Editorial overview: Engineered proteins as tools in structural biology**

Steyaert, J. & Yeates, T. O., Feb 2020, In: Current Opinion in Structural Biology. 60, p. V-VI

### **Nanobodies to study protein conformational states**

Uchański, T., Pardon, E. & Steyaert, J., Feb 2020, In: Current Opinion in Structural Biology. 60, p. 117-123 7 p.

### **Modulation of the Erwinia ligand-gated ion channel (ELIC) and the 5-HT3 receptor via a common vestibule site**

Brams, M., Govaerts, C., Kambara, K., Price, K. L., Spurny, R., Gharpure, A., Pardon, E., Evans, G. L., Bertrand, D., Lummis, S. C., Hibbs, R. E., Steyaert, J. & Ulens, C., 28 Jan 2020, In: eLife. 9, e51511.

**Binding domains directed against GPCR:G protein complexes and uses derived thereof**

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**Fusion protein with a toxin and scaffold protein**

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**Fusion proteins comprising a cytokine and scaffold protein**

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**Methods to select for agents that stabilize protein complexes**

Steyaert, J., Wohlkönig, A. & Triest, S., 2020, Patent No. US10641779

**Novel antigen-binding chimeric proteins and methods and uses thereof**

Steyaert, J., Pardon, E., Uchanski, T. & Vranken, W., 2020, Patent No. EP3704160

**Structural evidence for the critical role of the prion protein hydrophobic region in forming an infectious prion**

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**Structure of S-layer protein Sap reveals a mechanism for therapeutic intervention in anthrax**

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**A lipid site shapes the agonist response of a pentameric ligand-gated ion channel**

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**The morphogen Sonic hedgehog inhibits its receptor Patched by a pincer grasp mechanism**

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### **An improved yeast surface display platform for the screening of nanobody immune libraries**

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### **Structure of Prototypic Peptide Transporter DtpA from *E. coli* in Complex with Valganciclovir Provides Insights into Drug Binding of Human PepT1**

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