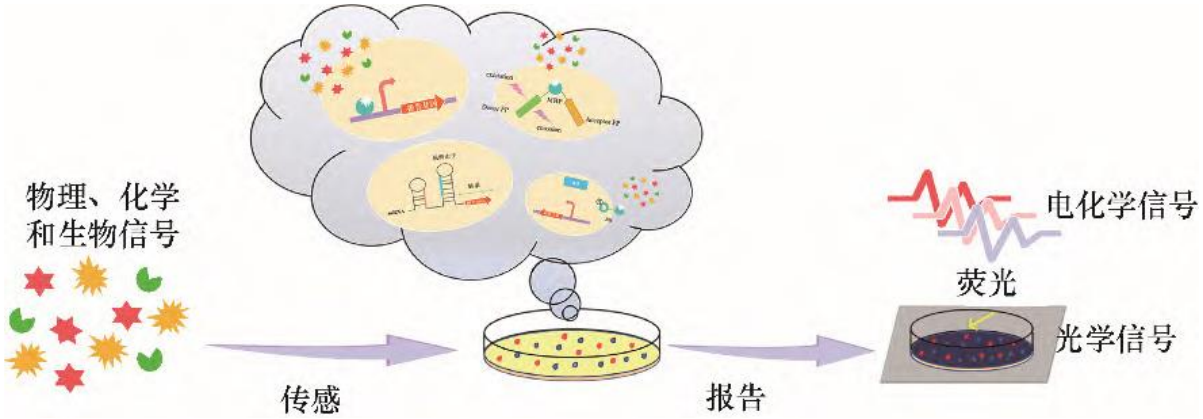
LOCKR-based *de novo* Protein Biosensor Design

分享人：张伟平

生物传感器(biosensor)



传感器种类：

- 转录调节元件
- 翻译调节元件
- 蛋白质

孙怡, 张腾, 吕波, 李春. 化工学报, 2022, 73(2): 521-534

蛋白质生物传感器的优点与面临的挑战

优点

1. **时滞低。**基于转录、翻译调节元件的传感器具有时滞性（转录/翻译花时间），不便于即时检测(point-of-care test)
2. **适应面广**。蛋白质传感器的活性一般取决于三维结构是否完整，而基于转录/翻译调节元件的传感器在不同物种内的活性相差较大。

挑战

1. **种类少。**基于蛋白质的生物传感器一般局限于对自然存在的蛋白质进行重新改造，不容易找到结合特定配体的蛋白质，即使找到了也需要考虑大量的因素以有效的将传感系统与报告系统耦合

孙怡, 张腾, 吕波, 李春. 化工学报, 2022, 73(2): 521-534

Quijano-Rubio, A., Yeh, HW., Park, J. *et al. Nature* **591,** 482–487 (2021)

Article

De novo design of modular and tunable protein biosensors

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Alfredo Quijano-Rubio[1,2,8], Hsien-Wei Yeh[1,8], Jooyoung Park[1,6], Hansol Lee[3], Robert A. Langan[1,7], Scott E. Boyken[1,7], Marc J. Lajoie[1,7], Longxing Cao[1], Cameron M. Chow[1], Marcos C. Miranda[1], Jimin Wi[4], Hyo Jeong Hong[4], Lance Stewart[1], Byung-Ha Oh[1,3]✉ & David Baker[1,5]✉

Naturally occurring protein switches have been repurposed for the development of biosensors and reporters for cellular and clinical applications[1]. However, the number of such switches is limited, and reengineering them is challenging. Here we show that a general class of protein-based biosensors can be created by inverting the flow of information through de novo designed protein switches in which the binding of a peptide key triggers biological outputs of interest[2]. The designed sensors are modular molecular devices with a closed dark state and an open luminescent state; analyte binding drives the switch from the closed to the open state. Because the sensor is based on the thermodynamic coupling of analyte binding to sensor activation, only one target binding domain is required, which simplifies sensor design and allows direct readout in solution. We create biosensors that can sensitively detect the anti-apoptosis protein BCL-2, the IgG1 Fc domain, the HER2 receptor, and Botulinum neurotoxin B, as well as biosensors for cardiac troponin I and an anti-hepatitis B virus antibody with the high sensitivity required to detect these molecules clinically. Given the need for diagnostic tools to track the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[3], we used the approach to design sensors for the SARS-CoV-2 spike protein and antibodies against the membrane and nucleocapsid proteins. The former, which incorporates a de novo designed spike receptor binding domain (RBD) binder[4], has a limit of detection of 15 pM and a luminescence signal 50-fold higher than the background level. The modularity and sensitivity of the platform should enable the rapid construction of sensors for a wide range of analytes, and highlights the power of de novo protein design to create multi-state protein systems with new and useful functions.

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Protein Design

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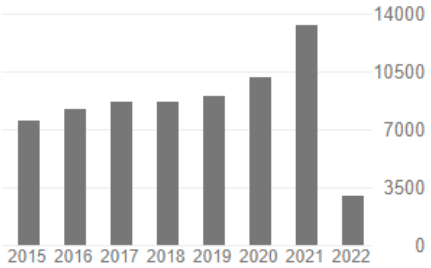

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Accurate prediction of protein structures and interactions using a three-track neural network

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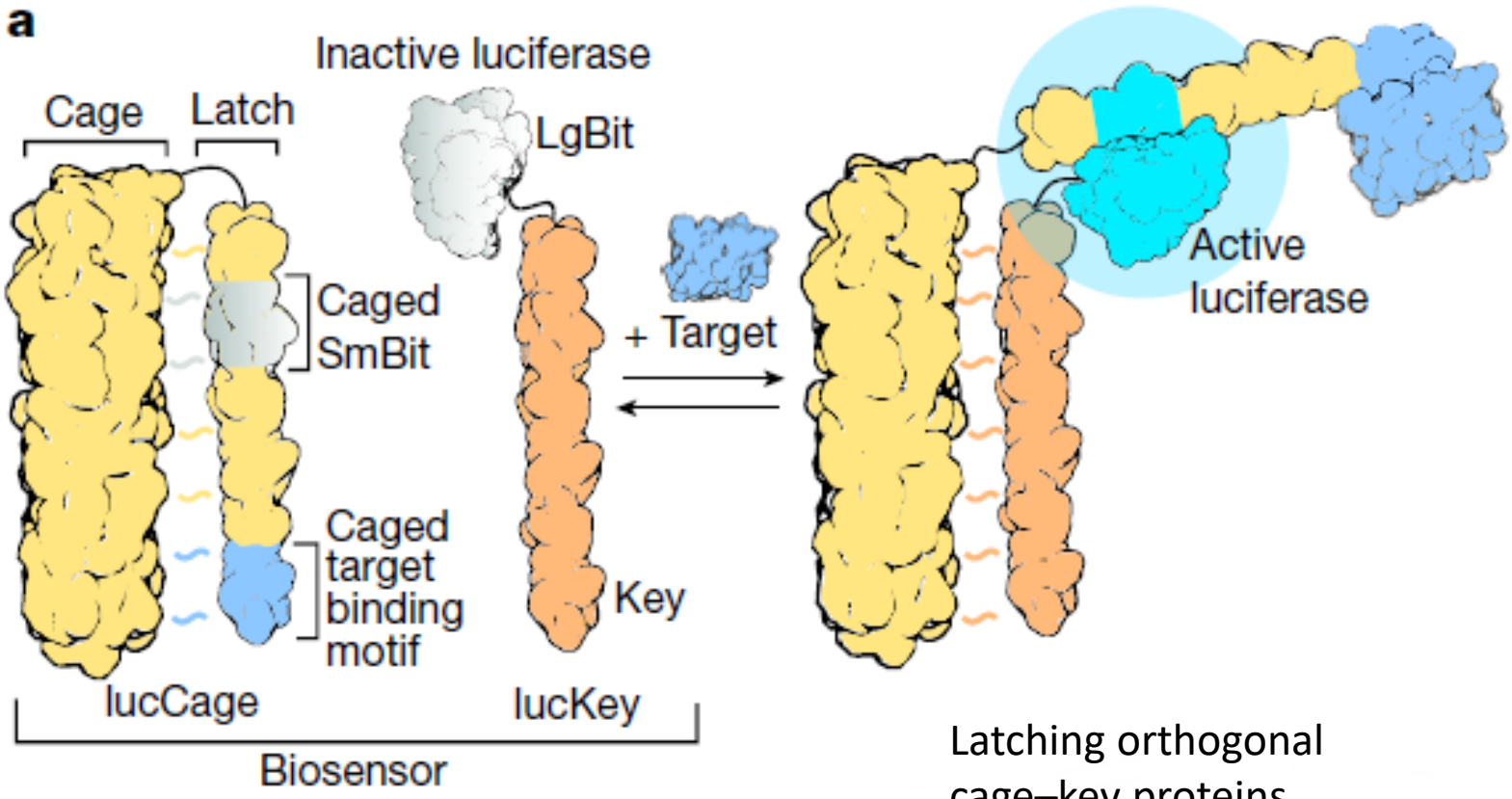
DeepMind presented remarkably accurate predictions at the recent CASP14 protein structure prediction assessment conference. We explored network architectures incorporating related ideas and obtained the best performance with a three-track network in which information at the 1D sequence level, the 2D distance map level, and the 3D coordinate level is successively transformed and integrated. The three-track network produces structure predictions with accuracies approaching those of DeepMind in CASP14, enables the rapid solution of challenging X-ray crystallography and cryo-EM structure modeling problems, and provides insights into the functions of proteins of currently unknown structure. The network also enables rapid generation of accurate protein-protein complex models from sequence information alone, short circuiting traditional approaches which require modeling of individual subunits followed by docking. We make the method available to the scientific community to speed biological research.

The comp. of LOCKR: LucCage, LucKey &Target

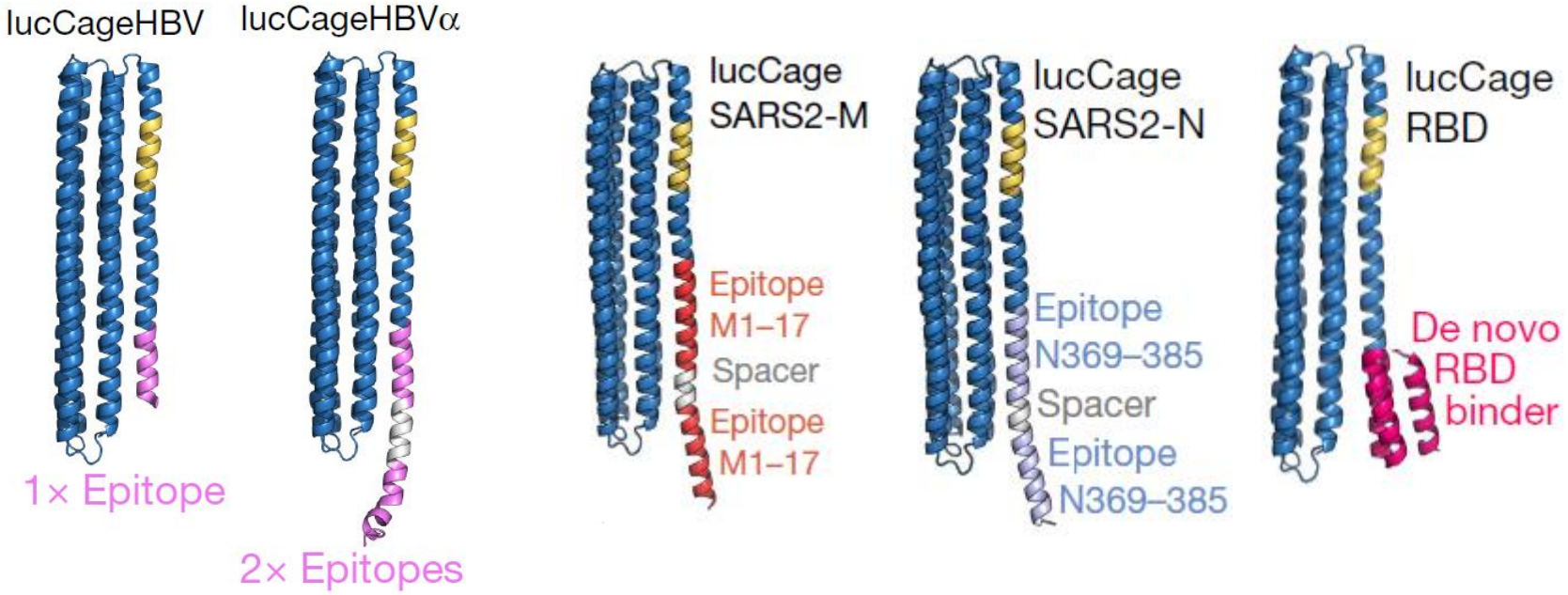


Latching orthogonal cage–key proteins (LOCKR)

Quijano-Rubio, A., Yeh, HW., Park, J. *et al. Nature* **591,** 482–487 (2021)

Modular?

1. 使用LucCage-LucKey系统设计新的生物传感器时，只需要设计“目标蛋白结合基序（target binding motif，TBM）”即可
2. TBM即可以是天然存在的蛋白结构域，也可以是基于结构预测的蛋白
3. Rosetta的GraftSwitchMover脚本可以帮助设计者“一步完成传感器设计”



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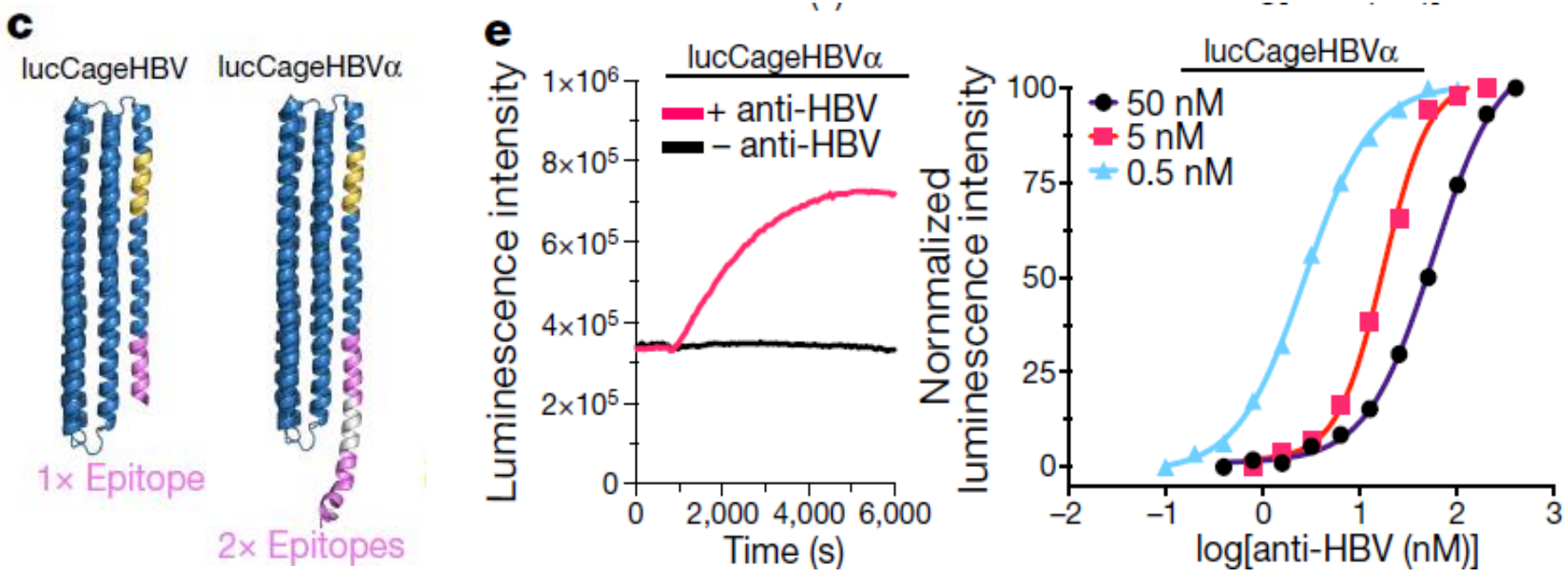
Tunable?



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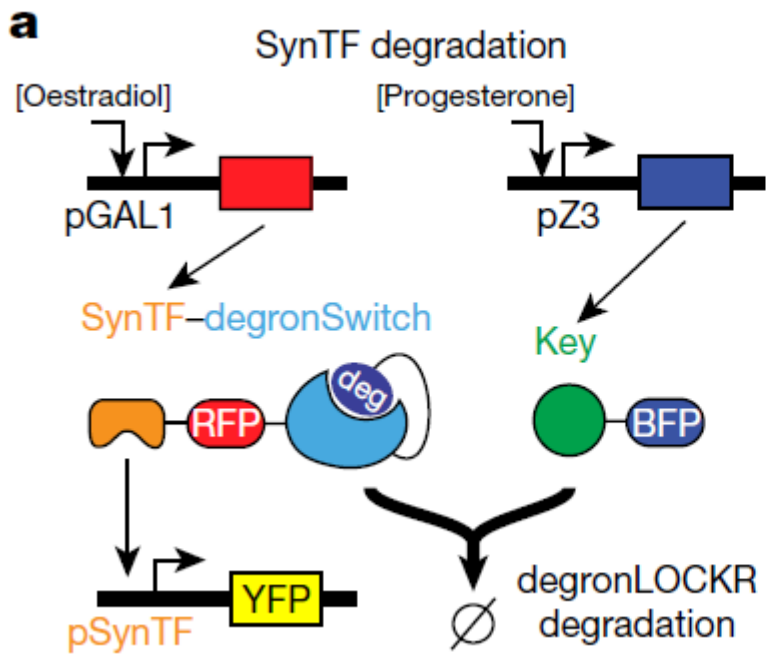
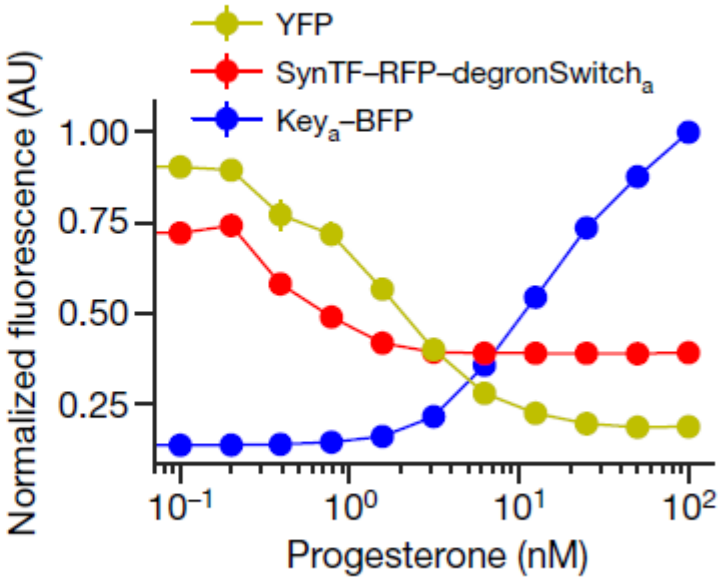
LOCKR的其他应用

1 通过关在笼子中的降解子(caged degron)调控基因表达（degronLOCKR）

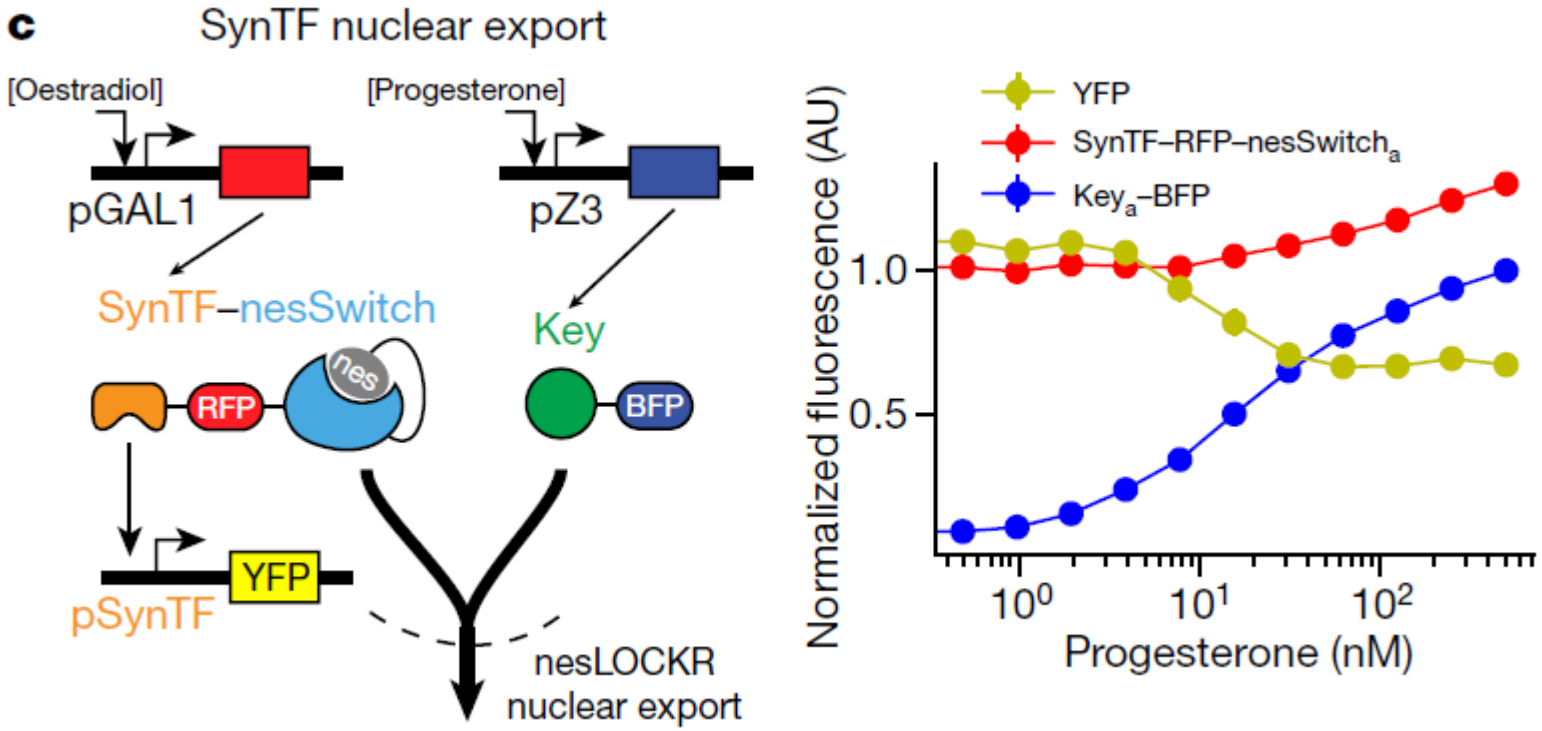




Langan, R.A., Boyken, S.E., Ng, A.H. *et al.* *Nature* **572,** 205–210 (2019).

LOCKR的其他应用

2. 通过关在笼子中的出核序列(caged nuclear export sequence)调控蛋白质的核内核外分布(nesLOCKR)



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"The potential of LOCKR is more than what is strictly published in this paper"

— Bobby Langan