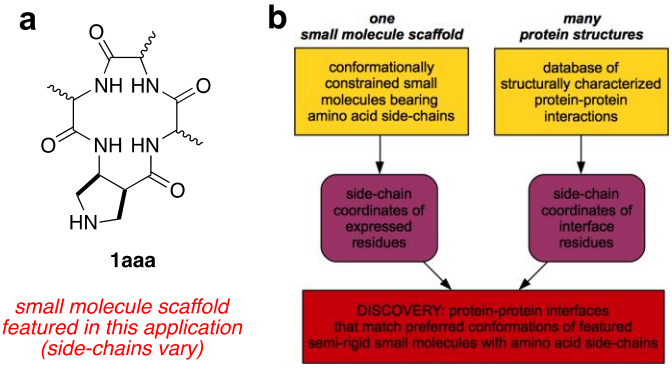
Research Projects: Novel Small Molecules To Bind Proteins

A. EKO To Discover Small Molecules That Perturb Protein-protein Interactions

EKO (DOI:10.1021/ja3067258) is a process conceived by Dr Burgess. It has several unique aspects. First, EKO requires special chemotypes, and these tend to be novel, patentable structures. Second, EKO uses the protein-*ligand* as a template for finding hits, unlike all other forms of virtual screening that use the protein-receptor to do this. Said differently, EKO copies keys (protein-ligands), whereas current virtual screening methods are based on finding molecules to fit locks (the protein-receptors).



Burgess *et al* experimentally validated the EKO approach by using it to develop inhibitors to dimerization of HIV-1 protease.[1] His group then validated the approach for another PPI featuring the serpin antithrombin.[2]

Fig 1. **a** Structure **1** conforms to the chemotype guidelines outlined below. **b** In EKO, preferred small molecules conformers are compared to PPI interfaces in the PDB.

Preferred EKO chemotypes have:

- at least three amino acid side-chains;
- kinetically and thermodynamically accessible conformations (*ie* not too rigid); and,
- moderate entropy loss on docking (*ie* only a few significant degrees of freedom that influence the side-chain orientations).

Compound **1** (Fig 1a) is illustrative of EKO chemotypes: it projects three amino acid side-chains in a limited range of orientations. Interaction energies in PPIs are most often based on side-chain to side-chain interactions (>88 %).[1,3,4] In EKO a semi-rigid small molecule scaffold is equipped with the same side-chains as a small interface segment in a PPI, and could present these in the same orientations as on the protein-ligand, and is then a candidate for disrupting that PPI.[5]

EKO is based on comparing side-chain orientations in structurally characterized PPIs with simulated conformers of the chemotypes (Fig 2a and b). This process reveals PPI regions for which the semi-rigid scaffold can display amino acid side-chains in almost the same way, implying it could perturb the interface by partly or wholly displacing one protein.

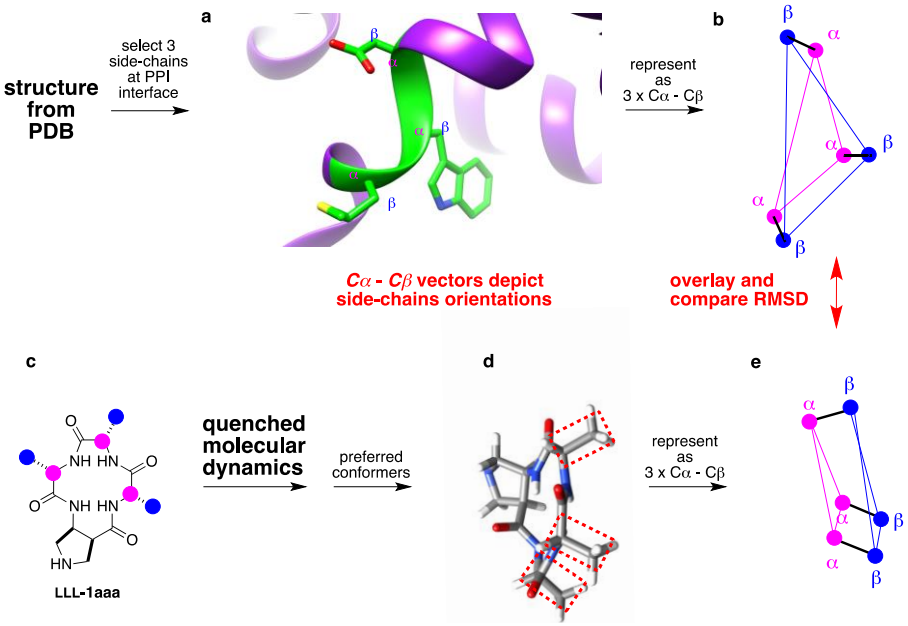


Fig 2. *Cα - Cβ* vectors: at PPI interfaces (**a** and **b**) and in preferred conformations of **1aaa** (**c – d**), **e** Libraries of these coordinates are overlaid, and goodness of fit is expressed as RMSDs (root mean square deviations, Å).

Preferred conformations in EKO and their side-chain orientations are simulated using the scaffold bearing only three methyl substituents. This is the simplest way to assess how the scaffolds tend to present *Cα* and *Cβ* coordinates *for any side chains* in the

absence of solvent and intramolecular side-chain-to-side-chain interactions. Accessible scaffold conformers may overlay well on *any* three interface-amino acid side-chains, and, if they do, this reveals *an accessible conformation that is reinforced by the protein-binding partner that accommodates the side-chains of one protein in the PPI*, *and should similarly accommodate the mimic.*

B. Small Molecules For Active Targeting In Cancer

Therapeutic indices are key pivotal in cancer chemotherapy: identifying cytotoxic substances is easy, but it is hard to find ones that selectively kill cancerous over healthy tissue. One way to increase therapeutic indices of cytotoxic substances is to attach an entity that will bind receptors selectively overexpressed on tumor cell-surfaces and, ideally, deliver cytotoxic cargos into the cell. *Active targeting*[6] like that is different to *molecularly targeted therapy* in which tyrosine kinase receptor inhibitors inhibit biochemical pathways upregulated in cancer cells.

$$\text{therapeutic index} = \frac{\text{toxic dose}}{\text{therapeutic dose}} \text{ or } \frac{\text{lethal dose}_{50}}{\text{effective dose}_{50}\ \textit{in animals}} \text{ or } \frac{\text{tolerated dose}_{50}}{\text{effective dose}_{50}\ \textit{in humans}}$$

Active targeting is non-trivial because the dense interstitial matrix of solid tumors presents a viscous, imposing barrier to diffusion of therapeutic agents. Solid stress in tumors compresses blood vessels within them, and this makes it particularly hard for nanoparticles, polymers, and large biomolecules to permeate.[7] Tumor vasculature tends to be immature, leading to reduced pressure gradients and heterogeneous blood flow,[8,9] while tumor hydrostatic pressure is high, disfavoring convective drug transfer from blood vessels. This is the physiological basis of the so-called EPR effect, and highlights the main problem with delivery of monoclonal antibodies (mAbs) and nanoparticles: accumulation of larger agents around the periphery of the tumor.[10]

Overexpression of the cell surface receptor tropomyosin-receptor-kinase C (TrkC) is characteristic of *metastatic* melanoma[11-19] and of *metastatic* breast cancer,[20-26] but not of non-metastatic forms of these diseases.† This is extremely important here because much of this application is about a small molecule fragment that seems to target TrkC, and has the potential to differentiate metastatic forms of these disease states from others that are non-lethal and need not be treated aggressively. Moreover, *the same molecule* has the potential to be used to image the extent of metastatic spread via positron emission tomography (PET), to treat the primary lesion via photodynamic therapy (PDT), and to restrict formation of metastases (Fig. 3). It is rare that a chemotherapeutic can be used as a stain in histology, and labeled to give an imaging agent without changing the molecular structure. Thus, at the same dose, the agent developed in this application to image TrkC$^+$ tumors have the same pharmaco-kinetic and -dynamic properties as the one used to treat them. This is not true of clinically established PET label/chemotherapy combinations, *eg* imaging probes such as FDG have different distribution profiles to chemotherapeutics like taxol, and neither of these agents actively target TrkC$^+$ metastatic melanoma. A few PET/PDT theranostics,[27-34] including one for breast cancer,[35] are known, but none of them bind TrkC, or selectively target *metastatic* breast cancer. None of these PET/PDT theranostics feature BODIPY-based photosensitizers that are at the core of this application.

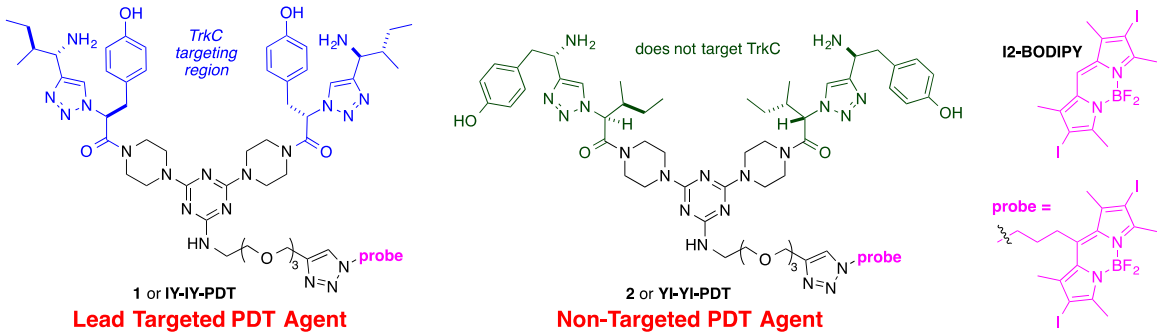


Fig 3. Lead targeting ligands.

Simultaneous with mAb development in oncology, there has been a swell of interest in multimodal nanoparticles, *because it is relatively easy to trap different agents in small grains of inert matrices.* However, there are many pharmacokinetic and toxicological issues associated with nanoparticles, large batch-to-batch variations, and almost insurmountable barriers to regulatory approval.

† Breast cancer is important here because this application will describe *in vivo* data for a TrkC-seeking agent *in a breast cancer model*, and propose obtaining similar data in melanoma models. The *in vivo breast* cancer model is relevant because it *features the same agent used to obtain the preliminary data described for melanoma cells and tissue slices*.

This work is significant because it describes *a discrete molecule* with potential for use in diagnosis, imaging, *and* treatment of $TrkC^+$ metastatic melanoma. Our team is uniquely placed to actively target TrkC having discovered a small molecule unit to do that. A broader impact of these studies is to expand the number of molecular fragments available for active targeting Trk expressing tumors, a type that is becoming recognized as prevalent in oncology.[36]

1. Exploring Key Orientations at Protein-Protein Interfaces with Small Molecule Probes, E. Ko, A. Raghuraman, L. M. Perez, T. R. Ioerger, and K. Burgess, *J. Am. Chem. Soc.*, 2013, **135**, 167-73.

2. Small Molecule Probes that Perturb a Protein-protein Interface in Antithrombin, D. Xin, A. Holzenburg, and K. Burgess, *Chem. Sci.*, 2014, **5**, 4914-21.

3. The Atomic Structure of Protein-Protein Recognition Sites, L. L. Conte, C. Chothia, and J. Janin, *J. Mol. Biol.*, 1999, **285**, 2177-98.

4. Modulators of Protein-Protein Interactions, L.-G. Milroy, T. N. Grossmann, S. Hennig, L. Brunsveld, and C. Ottmann, *Chem. Rev.* , 2014, **114**, 4695-748.

5. Minimalist and Universal Peptidomimetics, E. Ko, J. Liu, and K. Burgess, *Chem. Soc. Rev.*, 2011, **40**, 4411-21.

6. Passive and active drug targeting: drug delivery to tumors as an example, P. Torchilin Vladimir, *Handb. Exp. Pharmacol.*, 2010, **197**, 3-53.

7. Delivery of molecular and nanoscale medicine to tumors: transport barriers and strategies, V. P. Chauhan, T. Stylianopoulos, Y. Boucher, and R. K. Jain, *Annu. Rev. Chem. Biomol. Eng.*, 2011, **2**, 281-98.

8. Mosaic Tumor Vessels: Cellular Basis and Ultrastructure of Focal Regions Lacking Endothelial Cell Markers, E. di Tomaso, D. Capen, A. Haskell, J. Hart, J. J. Logie, R. K. Jain, D. M. McDonald, R. Jones, and L. L. Munn, *Cancer Res.*, 2005, **65**, 5740-9.

9. Abnormalities of basement membrane on blood vessels and endothelial sprouts in tumors, P. Baluk, S. Morikawa, A. Haskell, M. Mancuso, and M. McDonald Donald, *Am. J. Pathol.*, 2003, **163**, 1801-15.

10. Newly synthesized water soluble cholinium-purpurin photosensitizers and their stabilized gold nanoparticles as promising anticancer agents, D. Demberelnyamba, M. Ariunaa, and Y. K. Shim, *Int J Mol Sci*, 2008, **9**, 864-71.

11. Expression of neurotrophin receptor Trk-C in nevi and melanomas, X. Xu, S. R. Tahan, T. L. Pasha, and P. J. Zhang, *J. Cutan. Pathol.*, 2003, **30**, 318-22.

12. Expression and function of neurotrophins and their receptors in cultured human keratinocytes, A. Marconi, M. Terracina, C. Fila, J. Franchi, F. Bonte, G. Romagnoli, R. Maurelli, C. M. Failla, M. Dumas, and C. Pincelli, *J. Invest. Dermatol.*, 2003, **121**, 1515-21.

13. Human melanoma TrkC: its association with a purine-analog-sensitive kinase activity, D. Marchetti, B. Murry, J. Galjour, and A. Wilke-Greiter, *J. Cell. Biochem.*, 2003, **88**, 865-72.

14. Remodeling of the microenvironment by aggressive melanoma tumor cells, M. J. C. Hendrix, E. A. Seftor, D. A. Kirschmann, V. Quaranta, and R. E. B. Seftor, *Ann. N. Y. Acad. Sci.*, 2003, **995**, 151-61.

15. Expression and function of neutrophins and their receptors in human melanocytes, A. Marconi, M. C. Panza, M. Bonnet-Duquennoy, K. Lazou, R. Kurfurst, F. Truzzi, R. Lotti, G. De Santis, M. Dumas, F. Bonte, and C. Pincelli, *Int. J. Cosmet. Sci.*, 2006, **28**, 255-61.

16. Neurotrophins and Their Receptors Stimulate Melanoma Cell Proliferation and Migration, F. Truzzi, A. Marconi, R. Lotti, K. Dallaglio, L. E. French, B. L. Hempstead, and C. Pincelli, *J. Invest. Dermatol.*, 2008, **128**, 2031-40.

17. Neurotrophins in Skin Biology and Pathology, V. A. Botchkarev, M. Yaar, E. M. J. Peters, S. P. Raychaudhuri, N. V. Botchkareva, A. Marconi, S. K. Raychaudhuri, R. Paus, and C. Pincelli, *J. Invest. Dermatol.*, 2006, **126**, 1719-27.

18. Brain-metastatic melanoma: a neurotrophic perspective, D. Marchetti, Y. Denkins, J. Reiland, A. Greiter-Wilke, J. Galjour, B. Murry, J. Blust, and M. Roy, *Pathol. Oncol. Res.*, 2003, **9**, 147-58.

19. Brain metastases in melanoma: roles of neurotrophins, Y. Denkins, J. Reiland, M. Roy, N. D. Sinnappah-Kang, J. Galjour, B. P. Murry, J. Blust, R. Aucoin, and D. Marchetti, *Neuro-Oncology* 2004, **6**, 154-65.

20. A screen of the complete protein kinase gene family identifies diverse patterns of somatic mutations in human breast cancer, P. Stephens, S. Edkins, H. Davies, C. Greenman, C. Cox, C. Hunter, G. Bignell, J. Teague, R. Smith, C. Stevens, S. O'Meara, A. Parker, P. Tarpey, T. Avis, A. Barthorpe, L. Brackenbury, G. Buck, A. Butler, J. Clements, J. Cole, E. Dicks, K. Edwards, S. Forbes, M. Gorton, K. Gray, K. Halliday, R. Harrison, K. Hills, J. Hinton, D. Jones, V. Kosmidou, R. Laman, R. Lugg, A. Menzies, J. Perry, R. Petty, K. Raine, R. Shepherd, A. Small, H. Solomon, Y. Stephens, C. Tofts, J. Varian, A. Webb, S. West, S. Widaa, A. Yates, F. Brasseur, C. S. Cooper, A. M. Flanagan, A. Green, M. Knowles, S. Y. Leung, L. H. J. Looijenga, B. Malkowicz, M. A. Pierotti, B. Teh, S. T. Yuen, A. G. Nicholson, S. Lakhani, D. F. Easton, B. L. Weber, M. R. Stratton, P. A. Futreal, and R. Wooster, *Nat. Genet.*, 2005, **37**, 590-2.

21. TrkC signaling is activated in adenoid cystic carcinoma and requires NT-3 to stimulate invasive behavior, S. V. Ivanov, A. Panaccione, B. Brown, Y. Guo, C. A. Moskaluk, M. J. Wick, J. L. Brown, A. V. Ivanova, N. Issaeva, A. K. El-Naggar, and W. G. Yarbrough, *Oncogene*, 2013, **32**, 3698-710.

22. TrkC plays an essential role in breast tumor growth and metastasis, W. Jin, G.-M. Kim, M.-S. Kim, M.-H. Lim, C.-H. Yun, J. Jeong, J.-S. Nam, and S.-J. Kim, *Carcinogenesis*, 2010, **31**, 1939-47.

23. TrkC binds to the type II TGF-β receptor to suppress TGF-β signaling, W. Jin, C. Yun, M. K. Kwak, T. A. Kim, and S. J. Kim, *Oncogene*, 2007, **26**, 7684-91.

24. c-Src Is Required for Tropomyosin Receptor Kinase C (TrkC)-induced Activation of the Phosphatidylinositol 3-Kinase (PI3K)-AKT Pathway, W. Jin, C. Yun, J. Jeong, Y. Park, H.-D. Lee, and S.-J. Kim, *J. Biol. Chem.*, 2008, **283**, 1391-400.

25. TrkC: a new predictive marker in breast cancer?, M. J. Blasco-Gutierrez, I. J. San Jose-Crespo, E. Zozaya-Alvarez, R. Ramos-Sanchez, and N. Garcia-Atares, *Cancer Invest.*, 2007, **25**, 405-10.

26. Neurotrophins and their receptors in breast cancer, H. Hondermarck, *Cytokine Growth Factor Rev.*, 2012, **23**, 357-65.

27. Assessing the barriers to image-guided drug delivery, G. M. Lanza, C. Moonen, J. R. Baker, Jr., E. Chang, Z. Cheng, P. Grodzinski, K. Ferrara, K. Hynynen, G. Kelloff, Y.-E. K. Lee, A. K. Patri, D. Sept, J. E. Schnitzer, B. J. Wood, M. Zhang, G. Zheng, and K. Farahani, *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.*, 2014, **6**, 1-14.

28. Light-Triggered Theranostics Based on Photosensitizer-Conjugated Carbon Dots for Simultaneous Enhanced-Fluorescence Imaging and Photodynamic Therapy, P. Huang, J. Lin, X. Wang, Z. Wang, C. Zhang, M. He, K. Wang, F. Chen, Z. Li, G. Shen, D. Cui, and X. Chen, *Adv. Mater.* , 2012, **24**, 5104-10, S/1-S/18.

29. Transforming a targeted porphyrin theranostic agent into a PET imaging probe for cancer, J. Shi, T. W. B. Liu, J. Chen, D. Green, D. Jaffray, B. C. Wilson, F. Wang, and G. Zheng, *Theranostics*, 2011, **1**, 363-70.

30. The role of porphyrin chemistry in tumor imaging and photodynamic therapy, M. Ethirajan, Y. Chen, P. Joshi, and R. K. Pandey, *Chem. Soc. Rev.*, 2011, **40**, 340-62.

31. R. K. Pandey, N. S. James, Y. Chen, J. Missert, and M. Sajjad, "Bifunctional agents for imaging and therapy", Photodynamic Therapy: Methods and Protocols C. J. Gomer ed. 2010 Humana Press Inc.

32. Comparative Positron-Emission Tomography (PET) Imaging and Phototherapeutic Potential of 124I- Labeled Methyl- 3-(1'-iodobenzyloxyethyl)pyropheophorbide-a vs the Corresponding Glucose and Galactose Conjugates, S. K. Pandey, M. Sajjad, Y. Chen, X. Zheng, R. Yao, J. R. Missert, C. Batt, H. A. Nabi, A. R. Oseroff, and R. K. Pandey, *J. Med. Chem.*, 2009, **52**, 445-55.

33. Compared to Purpurinimides, the Pyropheophorbide Containing an Iodobenzyl Group Showed Enhanced PDT Efficacy and Tumor Imaging (124I-PET) Ability, S. K. Pandey, M. Sajjad, Y. Chen, A. Pandey, J. R. Missert, C. Batt, R. Yao, H. A. Nabi, A. R. Oseroff, and R. K. Pandey, *Bioconjugate Chem.*, 2009, **20**, 274-82.

34. Multimodality Agents for Tumor Imaging (PET, Fluorescence) and Photodynamic Therapy. A Possible "See and Treat" Approach, S. K. Pandey, A. L. Gryshuk, M. Sajjad, X. Zheng, Y. Chen, M. M. Abouzeid, J. Morgan, I. Charamisinau, H. A. Nabi, A. Oseroff, and R. K. Pandey, *J. Med. Chem.*, 2005, **48**, 6286-95.

35. TSPO 18 kDa (PBR) Targeted Photosensitizers for Cancer Imaging (PET) and PDT, Y. Chen, M. Sajjad, Y. Wang, C. Batt, H. A. Nabi, and R. K. Pandey, *ACS Med. Chem. Lett.*, 2011, **2**, 136-41.

36. TRKing Down an Old Oncogene in a New Era of Targeted Therapy, A. Vaishnavi, T. Le Anh, and C. Doebele Robert, *Cancer Discov.*, 2015, **5**, 25-34.