



UNIVERSITY OF OREGON

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April 1, 2013

Dr. Zoe Cournia
Biomedical Research Foundation
Academy of Athens
Division of Pharmacology-Pharmacotechnology
Athens, 11527
Greece

Dear Zoe,

I am pleased to write this letter to confirm our collaboration on designing a strategy to improve existing and discover novel inhibitors of Arp2/3 complex. We have been collaborating since early 2010 for the optimization of the existing inhibitors, CK-666 and CK-869, to compounds that have a higher binding affinity for the complex as well as the discovery of novel inhibitors of the Arp2/3 complex. This effort has produced on publication in *ChemMedChem*, where we optimized CK-666 to a novel compound with 3-fold improved potency. The compounds are tested for inhibition *in vitro* and *in vivo* in our laboratory. In this effort we recently joined forces with Prof. Levent Cavas and Prof. Yavuz Ergun from the Dokuz Eylul University in Izmir, Turkey, who perform the organic synthesis of optimized compounds and who have also provided us with a synthetic library of novel carbazole/carboxazole compounds as promising analogs of CK-666 and CK-869.

We routinely purify rabbit skeletal muscle actin, Arp2/3 complex, and Arp2/3 complex activators in our laboratory. We use fluorescent labeling of protein for multiple biophysical assays including the pyrenyl-actin polymerization assay. We have standardized this assay using a fluorescence plate reader so that we can simultaneously run twelve or more polymerization time courses, allowing us to rapidly generate inhibition curves showing the dependence of elongation rate on inhibitor concentration. This experimental setup will be quick and efficient in measuring the inhibitory effect of small molecules on actin polymerization. Moreover, we have developed an *in vivo* assay to probe the effect of the small molecule inhibitors in living cells. Our assay entails infection of human adenocarcinoma (AGS) cells with *Listeria monocytogenes* and measurement of the effect of the new compounds on comet tail formation in these cells. Cells will be treated with either inhibitor or with control compounds having similar structures but no inhibitory activity. Samples will then be fixed and stained with rhodamine-phalloidin to

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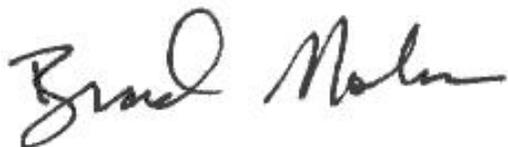
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visualize actin filaments using an epifluorescence microscope. The quantity of actin around each bacterium will be measured using an automation script in ImageJ image processing software as described. These experiments will be carried out in cell-culture facilities down the hall from our lab, and we will consult with Dr. Karen Guillemin, an expert in host-pathogen interactions and member of the Institute of Molecular Biology, on specific details of culturing, infecting and imaging cells.

We have solved several crystal structures of Arp2/3 complex with bound small molecule inhibitors, including a recent structure of CK-869 with *Bos taurus* Arp2/3 complex in press in *Chemistry and Biology*. Should additional structures provide insight into possible alterations for improved inhibition, we are well-suited to carry out these experiments. My extensive experience as an X-ray crystallographer, your expertise with computational approaches to lead compound optimization, along with our collaboration on the synthetic front will allow us to test computational predictions of ligand poses during each round of alteration. Starting with two distinct compounds with separate binding sites further improves our chances of improving the inhibitors through our iterative strategy.

This work is of significant biomedical importance given the role of Arp2/3 complex in metastasis of tumor cells and pathogen invasion of human cells. The proposed studies constitute a unique synergy between computer-aided drug discovery, organic synthesis, *in vivo* and *in vitro* assays, and X-ray crystallography. These experiments will at the very least lead to important new tools for studying the role of Arp2/3 complex in these processes, and at best, will prove useful for the treatment of human disease. I look forward to continuing our collaboration and to moving forward with the project.

Sincerely,

A handwritten signature in black ink, appearing to read "Brad Nolen". The signature is fluid and cursive, with the first name "Brad" being more prominent than the last name "Nolen".

Dr. Brad Nolen
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