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RECOLLECTION

From peptides to proteins: lessons from my years at the Centre for Protein Engineering

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The MRC Centre for Protein Engineering (CPE) hosted and trained many scientists over the years. It is a unique research environment that shaped the career of many scientists in all aspects. These include research directions and methodologies, but even more important—issues such as how to approach scientific problems and how to manage a research team. Alan Fersht was the director of the CPE when I joined it as a postdoc in the year 2000. In the current article for the PEDS special CPE issue, I will review how my scientific research and my approach to science developed from the days I arrived to the CPE as a young peptide chemist and throughout the years I spent at the CPE, and how it shaped my current research interests and attitude. I will focus on two major fields: (i) Using peptides to study and modulate the structure and interactions of proteins; (ii) Using quantitative biophysical methods to study proteins and their interactions at the molecular level.
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Introduction—or: why I went to the Centre for Protein Engineering

The connection between chemistry and biology is fascinating. The links between chemistry and biology are at all levels: the chemistry of biomolecules in living cells defines the process of life; chemical methodologies can be used as tools to study biological systems; however, most fascinating is the ability to rationally design molecules that modulate the activity of biological systems and may serve as drug leads. This can be achieved only based on comprehensive basic understanding of how the biological system of interest works at the molecular level.

Being made from the same natural building blocks that make proteins, peptides are useful tools to study and modulate biological processes (Benyamini and Friedler, 2010b). As a young PhD student in the laboratory of Prof. Chaim Gilon at the Institute of Chemistry, the Hebrew University of Jerusalem, I was intrigued by the question of how one can mimic active sites of proteins by cyclic peptides (Kasher *et al*., 1999). My work focused on developing cyclic peptides that mimic the conformation of the nuclear localization signals of HIV-1 proteins. These peptides inhibited nuclear import of the HIV-1 proteins MA and Tat *in vitro* and HIV-1 replication in cell culture (Friedler *et al*., 1998, 2000).

When the time came to look for a laboratory to do a postdoc, it was clear to me that in order to really understand proteins it is required to learn how to work with them. I needed to learn how to study proteins from all aspects, and thus contacted Prof. Alan Fersht at the Centre for Protein Engineering (CPE). This seemed like the best option to move from the peptide to the protein level and to learn how to apply quantitative biophysical methods to characterize proteins and their interactions. Indeed it was.

> ***Scene one: April 2000, Heathrow airport***
> A young couple and their one-and-a half years old child had just managed to stabilize their belongings on the stubborn trolley. The young man is a freshly graduated in chemistry. One of the couple's suitcases is leaving the trolley noisily and hitting the floor. The child is crying. The woman lets the man know that she has a better plan for carrying the entire luggage and that even though he has received his Ph.D degree he may still not know it all. The man does not answer, but in the coming years he will have this feeling (About not knowing it all, that is) more than often.

Chemical chaperones: shifting the folding equilibrium of p53

My research in the CPE combined the peptide world with the protein world. The idea was to develop chemical chaperones—peptides that refold and reactivate oncogenic mutants of the tumor suppressor protein p53. The p53 protein is at the center of the cellular network that protects organisms against cancer. It is a transcription factor that is induced upon oncogenic stress and activates genes that leads to cell cycle arrest or apoptosis, preventing the potential transformation into a cancer cell (Vousden and Prives, 2009). p53 is mutated in over half of human cancers (Brown *et al*., 2009). Almost all of these mutations are in the DNA-binding core domain of the protein (p53CD). Most p53 mutants are inactive due to loss of structure in the core domain, which can range from local unfolding at their DNA-binding interface to global denaturation (Bullock and Fersht, 2001). Reactivation of p53 mutants is a long-standing goal in cancer research (Brown *et al*., 2009).

Our project was based on a thorough quantitative research performed at the CPE in the late 1990s, which involved the expression and purification of numerous p53 core domain mutants and biophysical studies of their thermodynamic stability [see for example (Bullock *et al*., 1997, 2000; Nikolova *et al*., 1998, 2000)]. From these studies it was clear that in order

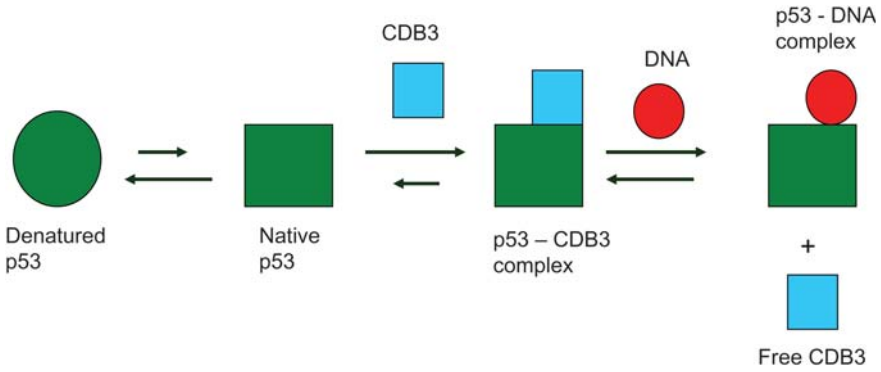

Fig. 1. Mechanism of action of CDB3, the peptidic chemical chaperone that refolds and reactivates oncogenic p53 mutants (from Friedler *et al*., 2002; ©PNAS).

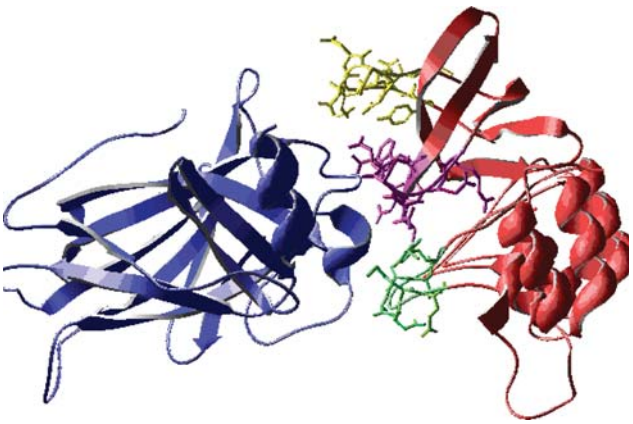

Fig. 2. The crystal structure of p53 core domain (blue) in complex with 53BP2 (red) (Gorina *et al*., 1996), based on which the CDB3 peptide (magenta) was designed. Figure taken from Friedler *et al*. (2002; ©PNAS).

to rescue such mutants, they needed to be refolded and restabilized (Bullock and Fersht, 2001). Our chaperone strategy involved the use of peptides that specifically bind to the native state of mutant p53 and shift the folding equilibrium of p53 towards it (Fig. 1). The peptides were designed based on protein–protein interactions of p53, since the p53-binding interfaces in its binding proteins were already optimized by nature to bind the core domain. We used the structure of the complex between p53CD and its binding protein 53BP2 (Gorina and Pavletich, 1996) [later known as a short variant of the ASPP2 protein (Samuels-Lev *et al*., 2001)] to design p53-stabilizing peptides (Fig. 2). We developed a peptide, CDB3 (Core Domain Binding peptide 3), which indeed acted as a chemical chaperone. The peptide restored the DNA-binding activity *in vitro* to p53 mutants (Friedler *et al*., 2002), refolded (Friedler *et al*., 2004) and reactivated p53 mutants *in vitro* (Friedler *et al*., 2002) and in cancer cells (Issaeva *et al*., 2003). Interestingly, we could show that the p53CD mutants are also kinetically destablizised compared with the wild type protein. They denatured much faster, and the CDB3 peptide also stabilized these mutants kinetically (Friedler *et al*., 2003).

Scene two: August 2002, Cambridge

Cambridge is going through a more-than-ever wet summer. The young scientist would not admit it to his wife yet (maybe he still remembers the scene at the airport), but cycling every day to the CPE and back does not seem the brightest idea any more. Coming to the CPE all the way from his homeland, on the other hand, was worth it all. To him the CPE is like a giant candy store to a child: full of state-of- the- art equipment, technical support as one could only imagine, and above all- lots of bright people to share ideas with. This whole wonderland is being conducted by Prof. Alan Fersht - A man to learn from.

Getting quantitative: how to really study protein–protein interactions

The most important concept I learnt early during my time at the CPE was the utmost importance of quantitative studies in all their aspects. The list of what 'quantitative' means in practice is very long, but includes among other things the use of quantitative biophysical methods instead of semi-quantitative biochemical methods, accurate data fitting, special attention to significant digits, accurate concentration measurements and accurate buffer preparation. I remember many of the group meetings where Alan went into the details of how the buffers were prepared, and was satisfied only when not only the pH, but also the ionic strength, was precisely controlled and known.

Quantitative analysis is critical for understanding biological processes. In the living cell, dynamic biological processes are mediated by changes in non-covalent interactions between biological molecules. Understanding these interactions at the molecular level is crucial for our understanding of how the living cells function. Moreover, impairment of protein–protein and protein–DNA interactions (e.g. owing to mutations such as in p53 or to bacterial or viral infection) can lead to severe diseases, such as cancer and AIDS. Understanding the molecular mechanisms leading to such impairment is essential in order to provide the basis for the development of drugs that will restore the impaired interactions or inhibit undesired interactions (e.g. with viral proteins). To identify drug targets and develop drugs against them it is crucial to gain quantitative understanding of the biological system of interest at the structural and molecular levels.

Biophysical methods are uniquely suited to quantitatively study the interactions between macromolecules in solution at the molecular level, and as such are essential to provide the basis for drug design. In the CPE, practically every biophysical method could be applied. It was indeed like a big

playground for a child—you could use every method you need. Both the equipment and, even more important, the expertise of how to use it in the best way, were always available. In my research, we used a variety of biophysical methods including NMR, fluorescence anisotropy, isothermal titration calorimetry, analytical ultracentrifugation, circular dichroism (CD) and numerous other methods. Combination of these methods with peptide chemistry, with a well-equipped protein expression and purification facility and—most important—the expertise and good will of highly qualified people who were always happy to help and teach—led to exciting, enjoyable and productive scientific research. In the CPE, the above methodology was used not only for stabilizing p53CD (see above), but also to study the mechanism of the interaction of p53 with many of its binding proteins, for example, MDM2 (Schon *et al*., 2002, 2004), Hif-1α (Hansson *et al*., 2002) and Rad51 (Friedler *et al*., 2005). The case of MDM2 is of special importance, since it is the main negative regulator of p53 and inhibition of this interaction is a key goal in cancer research. Understanding the molecular mechanism of the interaction between these proteins is essential for inhibitor design.

Scene three: MRC-LMB, the canteen. 12:45 P.M (Greenwich Mean Time)

Scientists originated from various places around the globe are experiencing ideas such as 'shepherds pie', 'fish and chips', 'chicken tikka masala', 'macaroni cheese' etc...Mind you - this is a serious business. Regularly, Alan's entire herd (including the shepherd himself) is eating there together. Sharing food consumption is accompanied by sharing scientific ideas as well as getting Alan's invaluable advise about topics such as which car to buy, which mortgage to choose and which hedge trimmer is the best.

Collaborating and sharing ideas

The CPE was a unique place for sharing ideas and expertise among scientists from all over the world. Each scientist in the CPE had his/her own unique expertise, which fit well within the general frame of the ongoing research. For example, some were expert in biophysics, some in NMR and some (like me) in peptides. Looking back, this is one of the major reasons for the success of the CPE. For interdisciplinary research that uses every possible methodology that would be required for addressing a scientific question, a crucial aspect is to have the right people with the right expertise. In my opinion, one of the greatest reasons for the success of the CPE is Alan's ability to constantly gather such a team of people who bring and share this expertise. From my personal point of view, there were several projects in the CPE to which I contributed peptide design and synthesis—for example (Allen *et al*., 2002; Seeliger *et al*., 2003; Ekblad *et al*., 2004).

Scene four (a comic relief): October 2004. Ben Gurion airport, Israel.

The former young couple is almost five years older now. One can spot another difference: they are now trying to balance twice the amount of suitcases on a trolley very similar to the Heathrow's shaky one. The world might have made a huge progress in the fields of communication technology, computer science and protein engineering, but trolley engineering and balancing suitcases are still unknown fields.

The not so young any more couple has now another child: A 4 years old girl. One of the suitcases is hitting the floor. The girl is crying. The woman is blaming the man... you know the script, don't you...?

So it is now the real world. In the real world there are peculiar concepts like 'research budget', 'grant writing', 'teaching', 'supervising students' and 'dry summer'.

How the CPE years shaped my independent research direction

In 2004, it was time to leave the CPE and move back home to start my independent position. It was really difficult to leave a place like the CPE, where it is possible to do practically every research one could dream of, and start everything from the beginning. Here again Alan was very clear—he said that it is important to leave at the right timing and move forward. Again, he was right.

Then, it came to the question of choosing an independent research direction. Naturally I chose directions that are based on what I learnt at the CPE and enjoy from both worlds: the peptide and the protein world. Our current research involves three major steps: (i) Analyzing protein–protein interactions in health, to understand how the particular biological system works at the molecular level; (ii) Understanding what goes wrong at the molecular level in disease, e.g. upon mutation or interaction with viral proteins; (iii) Development of drugs that will restore the biological system to its healthy conditions (in cancer), or will inhibit undesired interactions (in viral infection such as AIDS). To address these questions, we use an interdisciplinary approach combining peptide chemistry with what I learnt at the CPE: protein biochemistry and biophysical studies (e.g. fluorescence, CD and NMR spectroscopy). A significant part of our work involves the use of peptides to study protein–protein interactions (Benyamini and Friedler, 2010b). In particular two major lines of my current research were inspired by my CPE time:

(i) Shifting the oligomerization equilibrium of proteins as a therapeutic strategy: We developed a methodology, termed 'shiftides', to modulate the quaternary structure of proteins by shifting their oligomerization equilibrium using peptides that bind specifically to one oligomeric state. We have extended the chemical chaperones concept developed during my postdoc for p53 (Friedler *et al*., 2002) from modulating tertiary structure of proteins to modulating their quaternary structure in order to affect their function for therapeutic purposes (Hayouka *et al*., 2007). In more detail, many disease-related proteins are in equilibrium between active and inactive oligomeric states. Specific binding of peptides to one of the oligomeric states of such protein should result in shift of the equilibrium towards this state. We term peptides with such activity as 'shiftides', and they can be utilized therapeutically in two manners: (1) inhibiting a protein by shiftides that bind preferentially to its inactive oligomeric state and shift the oligomerization equilibrium towards it; (2) activating a protein by shiftides that bind preferentially to the active oligomeric state and shifting the oligomerization equilibrium towards it (Fig. 3). We

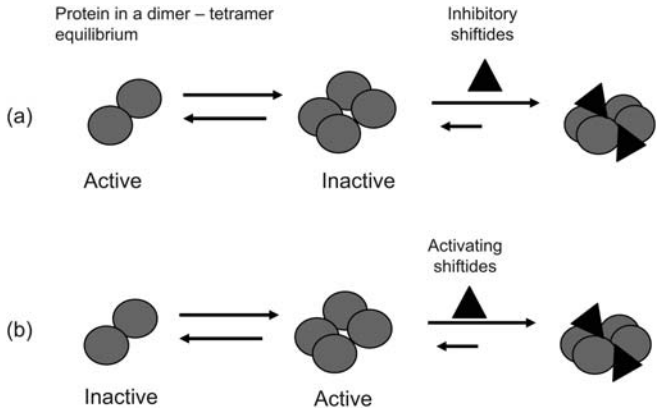

Fig. 3. Mechanism of action of shiftides: peptides that shift the oligomerization equilibrium of proteins. (**a**) protein-inhibitory shiftides; (**b**) protein-activating shiftides. The shiftides bind to one side of the oligomerization equilibrium and shift the equilibrium towards it. The shiftides are designed based on peptidic sequences from proteins that are known to bind a specific oligomeric form of the target protein.

applied this method for developing inhibitors of the HIV-1 integrase (IN) protein, which is a emerging target for anti-HIV drugs (Hayouka *et al*., 2007, 2010; Maes *et al*., 2009) as well as for other proteins such as non-muscle myosin II (Ronen *et al*., 2010).

(ii) Biophysical and structural studies of ASPP protein family. The ASPP proteins are apoptosis regulators: ASPP1 and ASPP2 promote, whereas iASPP inhibits, apoptosis (Slee and Lu, 2003; Sullivan and Lu, 2007; Rotem *et al*., 2007). In our lab we study the structure, interactions and regulations of the ASPP family members and mainly of the pro-apoptotic p53-binding protein ASPP2, which is responsible for the pro-apoptotic p53 response, and as such is involved in determining cell fate: survival or apoptosis. This is another connection to the CPE days: ASPP2 is the protein from which we derived the CDB3 peptide, which stabilizes p53 (see above) (Friedler *et al*., 2002). We studied the structure and interactions of ASPP2 using peptide arrays and biophysical methods. We found that the proline-rich domain of ASPP2 is natively unfolded and that it binds intramolecularly to the Ank-SH3 domains of the protein, which are responsible for its protein–protein interactions (Rotem *et al*., 2007, 2008). We also characterized the interaction of ASPP2 with NFkB as well as the Bcl2 family of anti-apoptotic proteins, revealed the binding sites and mechanisms and proposed a model for the biological role of the interactions (Katz *et al*., 2008; Benyamini *et al*., 2009). On the basis of these studies, we gained insight into the ASPP interaction network and suggested a role for electrostatics in differentiation between the pro- and anti-apoptotic family members (Benyamini and Friedler, 2010a).

Summary, or: what I learnt from Alan

The years at the CPE shaped my scientific career. Scientifically, I learnt there a whole lot of things: how to make and explore proteins, how to use biophysical methods, what is quantitative research and more. This was all detailed above. However, the most important thing I learnt from Alan at the CPE is how to approach science. Just a few examples: (i) in quantitative studies, always perform accurate and well-designed experiments and put an emphasis on correct data analysis. Understand the numbers and do not treat anything as 'black box'; (ii) the importance Alan gave to the 'work, finish, publish' idea. You should have a clear story and then publish it, not wait for ages; (iii) the importance of a paper is in how many times it is cited and not in where it is published; (iv) think broad—first ask a scientific question and then think which method is suitable to address it. If it is not set up in the lab—then set it up or find a good collaboration, but always use the most appropriate method to answer a given scientific question.

I also learned a good lesson in how to manage a research group. In the CPE there were more than 40 people at a given time during my days there, many more than in my research group, and still some principles are the same. Some examples: (i) give the students freedom but be there to guide them. Be their mentor and supervisor but do not tell them exactly what to do in every experiment. They should learn how to ask scientific questions and try to answer them: (ii) Do not look at how much time people spend in the lab but rather look at what they do at that time and judge them by the results they provide; (iii) Always be in constant touch with the group: Despite being very busy, Alan always had time for his group, had lunch with us every day (and afternoon tea) as well as weekly group meetings; (iv) If you are efficient, you can do an enormous amount of work. Try to be as efficient and 'to the point' as possible, and state clearly what you want.

Scene five: What my family knows - looking back. September 2010, home, Israel

Looking back- we all benefited from our days in Cambridge. My wife learnt to cycle (everything else she already knew) and I practiced the delicate English art of holding a full conversation about property prices, home improvement and the weather without doing anything about the topics mentioned. We had great time (and great conversations as well).

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Many thanks to Prof. Sir Alan Fersht for everything. Above all, for teaching me how to approach science and how to manage it, and for his enormous support all along the way. Thanks to all my former colleagues and friends at the CPE — it has been an amazing time. Thanks to all the current and former students in my laboratory. Big thanks to my wife and best friend, Dorit.

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