Molecular Recognition

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Molecular Mapping of the Recognition Interface between the Islet Amyloid Polypeptide and Insulin**

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The self-assembly of endogenous proteins into amyloid fibrils is the hallmark of a group of diverse human disorders including Alzheimer's disease, Parkinson's disease, and type II diabetes.[1] Many inhibition strategies have been developed in attempts to prevent this key process.[2] A leading approach is the search for natural agents that inhibit amyloid formation. Although most of the studied amyloidogenic proteins can readily form amyloid fibrils in vitro, they are normally stable and physiologically functional, even though there is no sequence diversity between healthy individuals and most of the affected patients. This raises the question as to what prevents these proteins from forming amyloid fibrils in normal situations. A possible explanation is the presence of natural stabilizing agents that prevent these aggregation-prone proteins from adopting the pathological amyloidal conformation. For example, the islet amyloid polypeptide (IAPP) hormone can rapidly form amyloid fibrils in vitro at concentrations that are 100-fold lower than those inside the secretory granules where it is stored. IAPP accumulates into amyloid deposits in the pancreas of type II diabetes patients, whereas in healthy individuals it is functional and soluble.[3] It has previously been shown that insulin is a highly efficient inhibitor of IAPP amyloid formation.[4] Insulin is colocalized and cosecreted with IAPP and it is suggested to stabilize IAPP inside the pancreatic cell. The physical interaction between IAPP and insulin has previously been characterized.[4] However, the molecular elements that mediate this highly important interaction remain to be identified.

In this study we used the natural inhibition mechanism that was selected by an elaborate evolutionary process as a basis to identify a novel and very effective inhibitory module. We performed a systematic reductionist analysis and used peptide-array methodology to identify the molecular determinants within insulin that facilitate the interaction with IAPP and vice versa.

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Insulin is composed of two chains, A and B, crosslinked by two disulfide bridges. As a first step, we evaluated the effect of each of the separated chains on IAPP amyloid formation. IAPP was allowed to form amyloid fibrils alone or in the presence of insulin chains. The fibrilization process was monitored by using the thioflavin T (ThT) fluorescence assay (see the Supporting Information). The inhibition abilities of human and bovine insulin were monitored as controls. Both bovine and human insulin exhibited a strong inhibitory effect on the aggregation of the highly amyloidogneic IAPP (Figure 1). The reductionist analysis allowed us to specifically

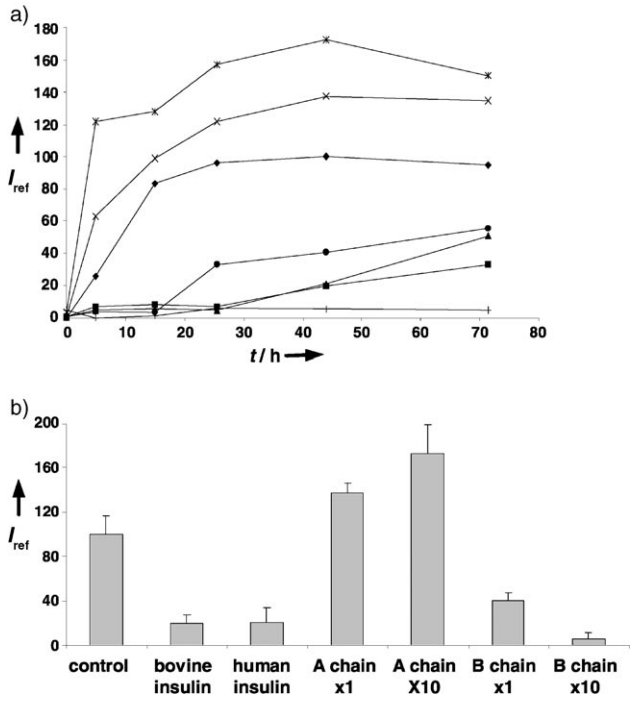

Figure 1. Influence of insulin chains on IAPP amyloid formation as assessed by a ThT-binding fluorescence assay. a) Kinetics of amyloid fibril formation by IAPP (◆) and by IAPP in the presence of bovine insulin (■), human insulin (▲), the insulin A chain at a onefold ratio (×) and at a tenfold ratio (*), and the insulin B chain at a onefold ratio (●) and at a tenfold ratio (+). Human and bovine insulin were added for comparison at a onefold ratio. Each graph represents the average of four independent repeats. b) Presentation of the relative fluorescence values I_{ref} of the samples after 44 h, when IAPP alone reached maximal ThT binding. Each column represents the average of four independent repeats; error bars represent the standard deviation.

pinpoint the B chain as a potent inhibitor which works in a dose-dependent manner. Its inhibition appears to be similar to that of the intact insulin molecule. At a 10-fold ratio, B-chain inhibition seems to be absolute throughout the course of the experiment. Conversely, no inhibition was observed in the presence of the A chain, but the aggregation was instead accelerated. Hence, we propose that the inhibition of IAPP by insulin is predominantly mediated by the B chain.

In order to confirm our assumption, we assessed the ability of the insulin chains to inhibit the IAPP structural transition from random coils into β sheets, a transition that is characteristic of amyloid formation, by using far-UV circular dichroism (CD) analysis (see the Supporting Information). IAPP was incubated alone or in the presence of the A chain, the B chain, or insulin at a 1:1 ratio. CD spectra were recorded after 0, 24, and 48 h (Figure 2).

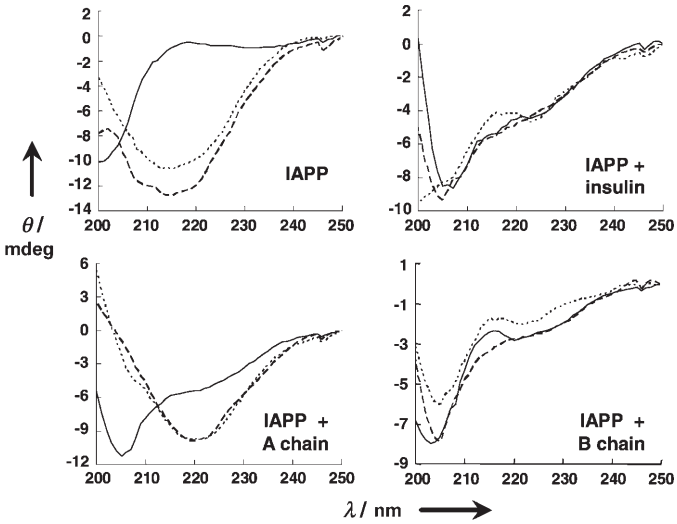

Figure 2. Influence of insulin chains on the IAPP structural transition. CD spectra (200–250 nm) of IAPP alone and in the presence of bovine insulin, the A chain, and the B chain. Changes in the IAPP secondary structure, as a result of amyloid formation, were tracked by measuring samples immediately after preparation (——), after 24 h (-----), and after 48 h (•••••).

The spectra of IAPP alone showed a clear transition from a random-coil conformation at zero time (minimum in the vicinity of 200 nm) to a β-sheet conformation (minimum in the vicinity of 217 nm) after 24 h. However, when the IAPP was incubated with insulin, this transition was not observed. This confirms the efficient insulin inhibition at a very early stage of the structural transition. The CD analyses of IAPP incubated with the B chain indicated a random-coil conformation without a transition over time, as observed in the presence of insulin. By contrast, when IAPP was incubated with the A chain, a structural transition was observed after 24 h, as in the case of IAPP alone. Accordingly, as observed for the formation of ThT-positive fibrils, only the B chain (and not the A chain) inhibits the process of structural transition by IAPP.

We further continued our reductionist analysis by mapping the recognition site within the B chain. To this end, we used an overlapping peptide array for a fine molecular mapping.[5] Briefly, 21 decamer peptides corresponding to consecutive overlapping fragments of the B chain were synthesized on a cellulose membrane. The membrane was incubated with biotinyl-IAPP, and IAPP-binding peptides were detected by dye precipitation with alkaline phosphatase conjugated avidin (see the Supporting Information). A large binding region was observed between residues 8 and 25 (Figure 3a). A second strong binding site was observed at the C-terminus spanning residues 20–30.

Next, we determined whether the peptide fragments of the B chain that bind IAPP are also capable of inhibiting

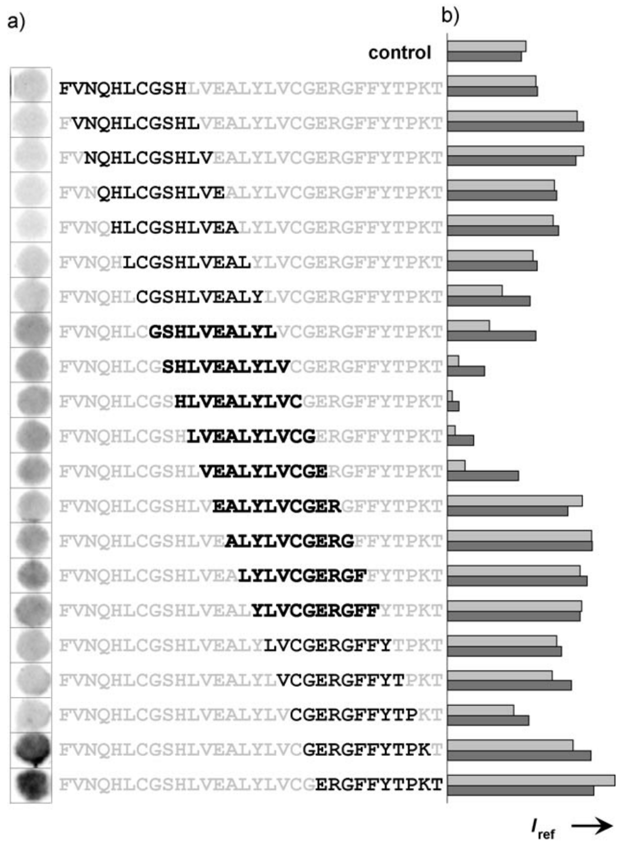

Figure 3. Screen for IAPP-binding and -inhibiting sequences within the insulin B chain. a) Decamer peptides corresponding to consecutive overlapping sequences of the B chain were synthesized on a cellulose membrane and incubated with biotinyl-IAPP. The sequence (black) of each spot and the position of each within the complete B chain sequence (gray) are presented. The sequences corresponding to the spots that exhibited binding are denoted in bold. b) The same peptides were cleaved from the membrane and then transferred onto a microtiter plate. Amyloid fibril formation by IAPP in the presence of the B chain peptides was assessed by the ThT-binding assay. Peptides were added at a 10-fold excess and ThT fluorescence was measured after 48 h (light gray) and 70 h (dark gray).

amyloid formation. We used ThT fluorescence as a complementary assay and searched for the inhibiting region within the B chain. IAPP fibrilization was performed in the presence of the same consecutive overlapping peptides, free in solution. Inhibition was observed with a set of peptides corresponding to residues 9–20 (Figure 3b). This region is included within the large region observed to bind IAPP. On the other hand, the peptides corresponding to the C-terminus sequence did not exhibit any inhibition effect. In addition, it was previously reported that insulin with a truncated B chain (lacking residues 23–30) also binds IAPP.[4e] Thus, the combination of the two assays points toward one central domain within the B chain that is responsible for binding to IAPP and preventing its aggregation.

To further pinpoint the region within IAPP to which insulin binds, we performed a reciprocal peptide-array analysis. 28 decamer peptides corresponding to consecutive overlapping fragments of IAPP were synthesized on a cellulose membrane and incubated with insulin (see the Supporting Information). A single binding domain spanning residues 7–19 was detected (Figure 4). Intriguingly, we previously identified this same region as the major recognition domain of IAPP fibrilization by using the same peptide-array method.[5] In addition, short peptide fragments derived from this region were found to associate into amyloid-like fibrils. Thus, insulin appears to interact with IAPP at the same region as that where IAPP self-interacts to form ordered amyloid assemblies.

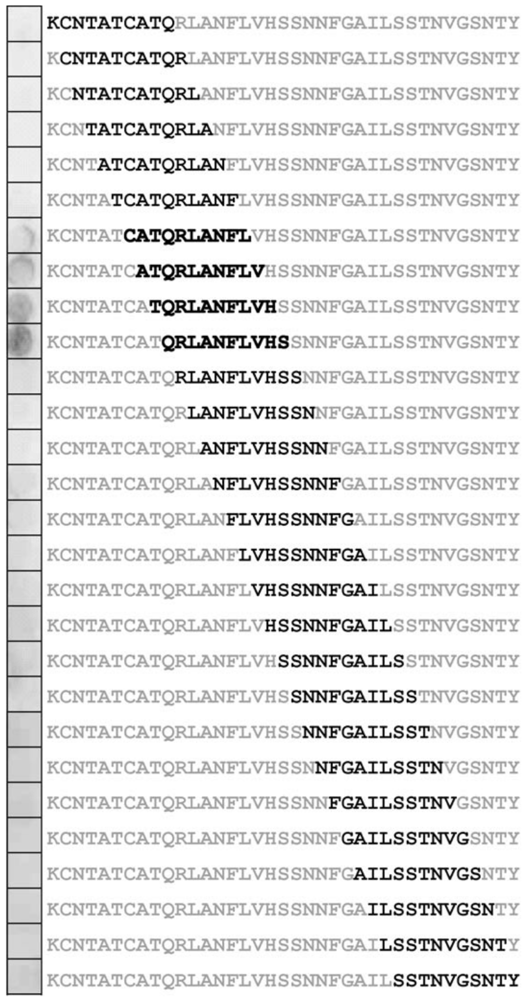

Figure 4. Screen for insulin-binding sequences within IAPP. Decamer peptides corresponding to consecutive overlapping sequences of IAPP were synthesized on a cellulose membrane and incubated with insulin. The sequence (black) of each spot and the position of each within the IAPP sequence (gray) are presented. Sequences corresponding to the spots that exhibited binding are denoted in bold.

To conclude, by a systematic biochemical reductionist approach we have identified a single domain within insulin that binds to IAPP and also inhibits IAPP amyloid formation. This domain is located at the center of the insulin B chain and spans residues 9–20. Moreover, we have identified a single domain within IAPP that binds insulin in the vicinity of residues 7–19. We propose that these two domains compose

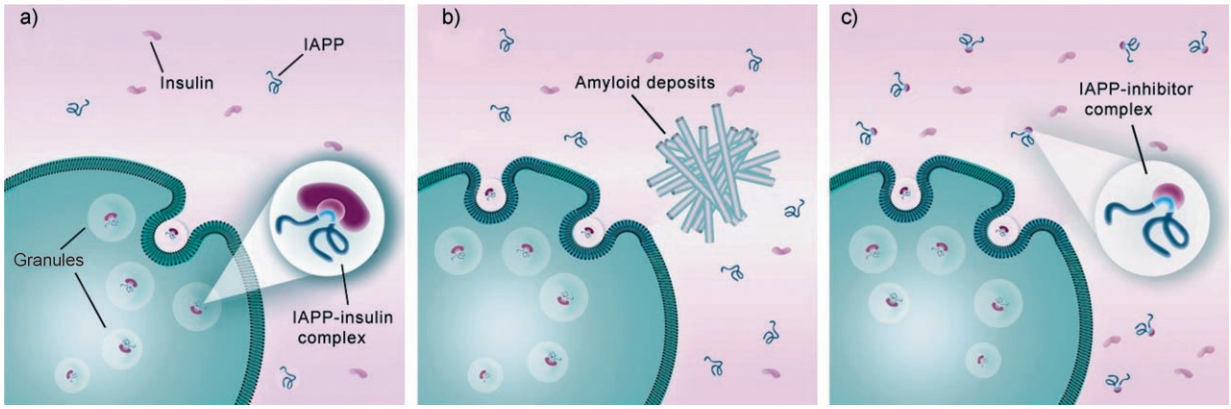

Figure 5. A possible model for IAPP and insulin behavior under different physiological conditions. a) In a healthy individual, IAPP and insulin form a complex within the secretory granules. When secreted, they disassociate and circulate in the blood. Random-coiled IAPP is shown in blue, globular insulin is shown in magenta. The magnification of the IAPP–insulin complex is depicted in the light circle with the recognition sites highlighted. b) In type II diabetes, secretion of IAPP and insulin is accelerated and the IAPP local concentration is increased. As a result, amyloid fibrils are deposited. c) Hypothetical strategy for treating type II diabetes: A short peptide, corresponding to the insulin recognition site, binds to secreted IAPP and prevents it from adopting the β-sheet conformation and accumulating into amyloid fibrils. The magnification of the IAPP–peptide inhibitor complex is depicted in the light circle.

the recognition interface that mediates the IAPP–insulin interaction.

Interestingly, the results are in good agreement with previous data and theories regarding amyloid formation and inhibition. First, as mentioned above, the identified domain within IAPP is the recognition site that is responsible for amyloid formation. It is very plausible that this domain will also be the target of insulin. Second, the two regions share sequence homology, as previously reported.[4e] The results directly support one of the proposals that was raised on the basis of sequence alignment. The two sequences are ANFLV, at positions 13–17 of IAPP, and ALYLV, at positions 14–18 of the insulin B chain. This homology probably allows the specific recognition between the domains. Third, according to the three-dimensional structure of insulin, the recognition region is organized in an α-helix conformation. It is possible that, in the context of the entire insulin molecule, this domain mediates inhibition by structure restriction, that is, by disabling the conversion into a β-sheet conformation. The β-breaking methodology is a central strategy of amyloid inhibition.[2b,c,f] We have previously exemplified this mechanism, when IAPP fibrilization was strongly inhibited by using α-aminoisobutyric acid (Aib) modified peptides, which also display a helical conformation.[6] Possibly, a similar mechanism underlies insulin inhibition. However, this suggestion should be further studied. Fourth, the identified region of the B chain contains an aromatic amino acid, tyrosine (at position 16), which is located opposite the phenylalanine residue of IAPP in the sequence alignment. We speculate that the tyrosine mediates IAPP–insulin recognition through π stacking with the phenylalanine. This assumption is based on our hypothesis regarding the key role of aromatic interactions in the process of amyloid formation[7] by providing directionality and stability to the assembled ultrastructural fibril.[8] Here, the tyrosine residue might play a similar role in the IAPP–insulin interaction.

Taken together, the results and characteristics discussed above clearly describe a putative molecular mechanism underlying insulin inhibition of IAPP fibrilization. Insulin binds to the amyloid-formation recognition site of IAPP through a specific recognition mechanism that is based on sequence homology and is possibly mediated by aromatic interactions. In this way, insulin blocks the ability of IAPP to assemble into amyloid fibrils through structure constraints.

Based on our conclusions, we suggest a hypothetical physiological mechanism for the IAPP–insulin interaction and a therapeutic approach of using insulin-derived peptides as amyloid inhibitors for type II diabetes (Figure 5). Within the secretory granule, insulin is found in 10–50-fold molar excess over IAPP. At this concentration ratio, kinetic inhibition is calculated to be maintained for several months, whereas the lifetime of a granule is several hours.[4d] This provides a reasonable explanation for the lack of amyloid fibrils inside the cell. When secreted, the IAPP–insulin complex dissociates, probably as a result of dilution beyond the association-constant concentration (Figure 5a). In the case of type II diabetes, the insulin and IAPP secretion is greatly accelerated due to insulin resistance. The amount of unbound IAPP outside the cell is augmented, which induces transient local high concentrations. As a result, IAPP associates into amyloid deposits (Figure 5b).

Due to the global prevalence of type II diabetes, there is a growing need to develop effective therapeutic agents to treat it. Since insulin is an excellent natural inhibitor, it may be very worthwhile to imitate its mechanism. We hypothesize that a properly designed peptide inhibitor, based on the insulin recognition module, might be an attractive drug lead for treating type II diabetes. Such a peptide inhibitor would bind to secreted IAPP in the same manner that insulin does and prevent its accumulation into amyloid fibrils (Figure 5c).

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[1] a) J. C. Rochet, P. T. Lansbury, Jr., *Curr. Opin. Struct. Biol.* **2000**, *10*, 60 – 68; b) C. Soto, *Nat. Rev. Neurosci.* **2003**, *4*, 49 – 60; c) C. M. Dobson, *Nature* **2003**, *426*, 884 – 890.

[2] a) L. O. Tjernberg, J. Näslund, F. Lindqvist, J. Johansson, A. R. Karlström, J. Thyberg, L. Terenius, C. Nordstedt, *J. Biol. Chem.* **1996**, *271*, 8545 – 8548; b) C. Soto, E. M. Sigurdsson, L. Morelli, R. A. Kumar, E. M. Castano, B. Frangione, *Nat. Med.* **1998**, *4*, 822 – 826; c) T. L. Lowe, A. Strzelec, L. L. Kiessling, R. M. Murphy, *Biochemistry* **2001**, *40*, 7882 – 7889; d) L. A. Scrocchi, Y. Chen, S. Waschuk, F. Wang, S. Cheung, A. A. Darabie, J. McLaurin, P. E. Fraser, *J. Mol. Biol.* **2002**, *310*, 697 – 706; e) H. M. Petrassi, S. M. Johnson, H. E. Purkey, K. P. Chiang, T. Walkup, X. Jiang, E. T. Powers, J. W. Kelly, *J. Am. Chem. Soc.* **2005**, *127*, 6662 – 6671; f) L. M. Yan, M. Tatarek-Nossol, A. Velkova, A. Kazantzis, A. Kapurniotu, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 2046 – 2051.

[3] a) P. Westermark, C. Wernstedt, E. Wilander, K. Sletten, *Biochem. Biophys. Res. Commun.* **1986**, *140*, 827 – 831; b) A. Kapurniotu, *Biopolymers* **2001**, *60*, 438 – 459; c) E. T. Jaikaran, A. Clark, *Biochim. Biophys. Acta* **2001**, *1537*, 179 – 203; d) A. E. Butler, J. Jang, T. Gurlo, M. D. Carty, W. C. Soeller, P. C. Butler, *Diabetes* **2004**, *53*, 1509 – 1516.

[4] a) P. Westermark, Z. C. Li, G. T. Westermark, A. Leckstrom, D. F. Steiner, *FEBS Lett.* **1996**, *379*, 203 – 206; b) S. Janciauskiene, S. Eriksson, E. Carlemalm, B. Ahren, *Biochem. Biophys. Res. Commun.* **1997**, *236*, 580 – 585; c) Y. C. Kudva, C. Mueske, P. C. Butler, N. L. Eberhardt, *Biochem. J.* **1998**, *331*, 809 – 813; d) J. L. Larson, A. D. Miranker, *J. Mol. Biol.* **2004**, *335*, 221 – 231; e) E. T. Jaikaran, M. R. Nilsson, A. Clark, *Biochem. J.* **2004**, *377*, 709 – 716.

[5] Y. Mazor, S. Gilead, I. Benhar, E. Gazit, *J. Mol. Biol.* **2002**, *322*, 1013 – 1024.

[6] S. Gilead, E. Gazit, *Angew. Chem.* **2004**, *116*, 4133 – 4136; *Angew. Chem. Int. Ed.* **2004**, *43*, 4041 – 4044.

[7] E. Gazit, *FASEB J.* **2002**, *16*, 77 – 83.

[8] a) R. Azriel, E. Gazit, *J. Biol. Chem.* **2001**, *276*, 34156 – 34161; b) S. Jones, J. Manning, N. M. Kad, S. E. Radford, *J. Mol. Biol.* **2003**, *325*, 249 – 257; c) G. G. Tartaglia, A. Cavalli, R. Pellarin, A. Caflisch, *Protein Sci.* **2004**, *13*, 1939 – 1941; d) O. S. Makin, E. Atkins, P. Sikorski, J. Johansson, L. C. Serpell, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 315 – 320; e) A. P. Pawar, K. F. Dubay, J. Zurdo, F. Chiti, M. Vendruscolo, C. M. Dobson, *J. Mol. Biol.* **2005**, *350*, 379 – 392; f) C. Wu, H. Lei, Y. Duan, *Biophys. J.* **2005**, *88*, 2897 – 2906; g) H. Inouye, D. Sharma, W. J. Goux, D. A. Kirschner, *Biophys. J.* **2006**, *90*, 1774 – 1789; h) F. G. De Felice, M. N. Vieira, L. M. Saraiva, J. D. Figueroa-Villar, J. Garcia-Abreu, R. Liu, L. Chang, W. L. Klein, S. T. Ferreira, *FASEB J.* **2004**, *18*, 1366 – 1372; i) Y. Porat, Y. Mazor, S. Efrat, E. Gazit, *Biochemistry* **2004**, *43*, 14454 – 14462.

