



Evaluation of biosensor surfaces for the detection of microtubule perturbation

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ABSTRACT

Dual polarization interferometry (DPI) and resonant mirror (RM) methods were used to characterize the growth of microtubules (MTs) on biosensor surfaces. The structure and dynamics of MTs play an important role in cell division and are a target for many anti-cancer drugs. Evidence from DPI demonstrated the growth of MTs on streptavidin–biotinylated-tubulin surfaces from the increase in mass and thickness, with a simultaneous decrease in density. The initial increase in thickness of 0.236 nm/min suggested the elongation of protofilaments before they join laterally to form the MT, where the rate of growth increased to 0.436 nm/min. Continuous mass increases were also observed when tubulin was added to a similar underlying RM surface. Tubulin binding to these surfaces was also temperature dependent, increasing the absolute response with MT stabilizers, while inhibiting binding with destabilizers when temperature was changed from 15 to 37 °C. Finally, the initial rates of tubulin assembly (mean ± SD, $n = 3$) with MT-stabilizer agents were significantly higher at 1.50 ± 0.27 and 1.04 ± 0.13 arcseconds/s, respectively, compared to 0.37 ± 0.11 arcseconds/s for tubulin containing GTP only. In the presence of the MT destabilizers, colchicine and dolastatin 10, the slopes of initial rates were lower than in their absence at 0.05 ± 0.01 and 0.27 ± 0.08 arcseconds/s, respectively. This provides evidence for the ability of surface-based optical sensors to distinguish between MT stabilizers and destabilizers, while also paving the path to develop other methods to screen for MT-perturbing agents using the same underlying surface engineering.

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1. Introduction

Biosensors have many applications in solving biological problems from macromolecular interactions (Lillis et al., 2006) to the binding of small molecules to surfaces and immobilized large molecules (Karlsson, 2004; Boozer et al., 2006). There are several types of instruments available that use different detection methods, each possessing their own advantages and disadvantages. Dual polarization interferometry (DPI) is a technique that utilizes a waveguide structure to detect changes on a sensing layer in real time. The waveguide consists of dielectric reference and sensing layers separated by a layer of cladding in such a manner that mimics Young's 2-slit experiment in optics (Cross et al., 2004). Light from a laser passes through the sandwiched waveguide structure and an interference pattern is detected on the opposing side by a camera. Any changes in refractive index that take place on the sensing layer alter the phase position of the fringes detected in real time, relative to the reference channel (Cross et al., 2004). Unlike the widely known surface plasmon resonance (SPR) tech-

nique (Homola, 2003), which utilizes only the transverse magnetic (TM) polarization of light, DPI takes advantage of measuring both the TM and transverse electric (TE) polarizations (Cross et al., 2004; Swann et al., 2004). Since there is only one unique solution that satisfies both the TM and TE when solving Maxwell's equations of electromagnetism, the final outcome is an effective refractive index and thickness (with precision on the order of 0.01 nm) (Cross et al., 2004), rather than relative values. Consequently, mass and density can be calculated for the layer throughout the experiment. This type of detailed information can be very helpful during the characterization of conformational changes during protein-small molecule interactions (Swann et al., 2003) and the design of surfaces for optical biosensors (Popplewell et al., 2009).

The resonant mirror (RM) method is similar to the SPR technique, but RM waveguide structures are capable of producing sharper resonance peaks than SPR and therefore enhance sensitivity (Lukosz, 1991). This is achieved by replacing the metal layer used in SPR with a high-index dielectric resonant layer. As light passes through a prism to a low-index medium it couples with the high-index resonant layer, thereby allowing total internal reflection to occur at the boundary of the sensing layer. Resonance occurs only when the angle of the incident light and the resonant modes in the high-index layer are phase-matched. Any change in the refractive

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index (density) of the biological layer at the surface corresponds to a change in the angle of light that satisfies this resonance condition (Nellen and Lukosz, 1991; Cush et al., 1993). Although DPI provides more detailed information of samples on the sensing surface, its current flow-through configuration consumes a much larger amount of sample in comparison with RM's small volume (microliter) cuvette. The RM cuvettes are also re-usable for multiple experiments.

Tubulin in its soluble form is a heterodimer of tightly bound α and β isotypes (Burns, 1991). Each isotype is a globular protein with molecular weight of ca. 50 kDa. The α subunit binds guanosine triphosphate (GTP) in a non-exchangeable manner, while GTP bound to the β subunit can hydrolyze to guanosine diphosphate (GDP) (MacNeal and Purich, 1978). Tubulin heterodimers assemble vertically to form protofilaments. Approximately 13 protofilaments join laterally in a three-start left-handed helix to form a hollow tube, the microtubule (MT) (Wade and Chretien, 1993). The presence of GTP plays an important role in the stability of the MT; heterodimers bound to GDP tend to make MTs unstable and, therefore, prone to disassembly (MacNeal and Purich, 1978). MTs provide cells with a scaffold for signaling proteins, as well as a railway for molecular motor proteins to perform their trafficking duties. MTs are also an essential component of the mitotic spindle, the apparatus that segregates sister chromatid pairs during cell division (Jordan and Wilson, 1998). These roles are a function of microtubule dynamics (Saxton et al., 1984), the relative rates of assembly and disassembly at both the plus and minus ends of the MTs. Increased MT dynamics is a hallmark of cancer cells (Jordan and Wilson, 2004). There are many MT-stabilizing and -destabilizing drugs that have been demonstrated to suppress cancer growth and cause cell death (Jordan and Wilson, 2004). For example, paclitaxel (Taxol®), a known natural product anti-cancer drug, has been demonstrated to stabilize MTs (Kumar, 1981), even in the absence of GTP (Howard and Timasheff, 1998). Epothilone B is another natural product that stabilizes MTs (Hamel, 2003), while other products such as dolastatin 10 (Hamel, 1992) and colchicine (Bhattacharyya et al., 2008) have a negative effect on MTs by destabilizing their formation. As many of the current antimicrotubule drugs have undesired toxicities, and/or cancer cells may become resistant to such drugs by increasing expression of multidrug resistance-associated proteins (Drukman and Kavallaris, 2002), it is, therefore, critical to screen for other agents, their analogs, and mixtures of agents that perturb MTs, but with fewer undesired toxicities. The structural changes that take place upon binding of MT-perturbing agents to tubulin vary with the type and location of tubulin binding (Sackett, 1995). An important step towards understanding the mechanism of such compounds depends on detecting the structural changes that take place upon binding. The ability to grow MTs on surfaces is also an important step in the development of high-throughput (e.g., microtiter plate-based or microarray) assays to monitor MT polymerization in the presence of various de-/stabilizer agents. We report a novel method to polymerize MTs on biosensor surfaces and demonstrate the ability to distinguish the interaction of stabilizers from destabilizers on MTs using a combination of RM and DPI, a major advance on previous work where the binding of MT inhibitors to tubulin immobilized onto SPR surfaces has been reported (Aoyama et al., 2007).

2. Materials and methods

2.1. Tubulin biotinylation

Tubulin, purified from bovine brain to electrophoretic homogeneity (Hamel and Lin, 1984), was biotinylated with 20-fold molar excess (50 mM) NHS-LC-biotin (Pierce, Chester, UK) diluted

in dimethylsulfoxide (DMSO) (Sigma, UK) following the manufacturer's procedures in 0.1 M 2-(N-morpholino)ethanesulfonic acid (MES) (USB, Affymetrix Inc., USA) (pH 6.9). Samples were then incubated on ice in a cold room for 2 h. Unreacted biotin was removed by centrifugation in a 30 kDa Nanosep (PALL Corp., Portsmouth, Hants, UK) microcentrifuge tube four times for 4 min at $5000 \times g$ and 4°C using a Jouan BR4i AB-2.14 rotor, washing the sample with 0.1 M MES buffer after each spin. The sample was then collected and stored at -80°C . The concentration of the biotinylated protein was determined with a bicinchoninic acid protein assay kit (Sigma, USA).

2.2. In vitro microtubule polymerization assay

MT polymerization was measured by the change in absorbance of the solution at 340 nm with a SpectraMax® M5 Microplate Reader (MDS Analytical Devices (US) Inc., Sunnyvale, California). Tubulin (or biotinylated-tubulin) (1 mg/mL) in 1 M monosodium glutamate (MSG) (USB, Affymetrix Inc., USA) (pH 6.6) with GTP (USB, Affymetrix Inc., USA) hereafter denoted as sample buffer, at 37°C was used, and positive and negative controls included $10\ \mu\text{M}$ paclitaxel and colchicine, respectively.

2.3. Functionalization of sensor surfaces

2.3.1. Dual polarization interferometry biosensor

The AnaLight® instrument (Farfield Group Ltd., Crewe, UK) was used for these experiments. NHS-LC-biotin (Pierce, Chester, UK) (200 mM in DMSO) was dropped onto the channels of an Amine Anachip™ (Farfield Group Ltd., Crewe, UK) *ex situ* for 10 min. The channels were then rinsed with deionized water before inserting into the chip manifold. With 0.1 M MES (pH 6.9) as the running buffer, the chip was calibrated with 4:1 ethanol–water (w/w) injections, as described (Swann et al., 2003), with the instrument set at 37°C . Streptavidin was injected at $35\ \mu\text{L}/\text{min}$ for 4 min followed by an injection of biotinylated tubulin at the same rate and time period. After each injection, the flow was returned to running buffer for a given time period so that unbound molecules were washed away and baseline was stabilized.

2.3.2. Resonant mirror biosensor

The IAsys instrument (NeoSensors, Ltd., UK) was utilized for these studies. A biotin cuvette (NeoSensors, Ltd., UK) was used as the base for the biotin-tubulin surface. The volume of sample used during all steps of the experiment was retained to $30\ \mu\text{L}$. Streptavidin (Sigma, UK) ($50\ \mu\text{g}$) was added to a cuvette containing 10 mM phosphate buffer (pH 7.7) and incubated for approximately 20 min or until a response of ~ 2000 arcseconds was obtained ($1\ \text{arcsecond} = 1/3600^\circ$; 600 arcseconds corresponds to $1\ \text{ng}/\text{mm}^2$ protein on the sensor surface). The phosphate buffer was removed from the cuvette, which was then washed and filled with 1 M MSG (pH 6.6) buffer. Approximately 50–60 μg of biotinylated tubulin was added to the cuvette for 10–15 min and the bound tubulin was washed with MSG buffer. For re-use, cuvettes were stripped with 12.5 M KOH for 20 s, which removes all the bound streptavidin and rinsed before a new layer of streptavidin was formed.

2.4. Microtubule polymerization on surfaces

2.4.1. Dual polarization interferometry biosensor

An injection of sample buffer was performed at $35\ \mu\text{L}/\text{min}$ for 2 min to subtract the relative bulk shift due to MSG when the sample was injected. Tubulin (1 mg/mL) in sample buffer was then injected continuously at $35\ \mu\text{L}/\text{min}$ for 45 min by replacing the standard sample loop with a 5 mL sample loop.

2.4.2. Resonant mirror biosensor

After the addition of biotinylated tubulin, the cuvette was washed with sample buffer. Approximately 70 μg of tubulin was then added to the cuvette and the change in response was monitored. When testing temperature dependence and the effect of compounds on initial rates of assembly, a mixture of sample buffer $\pm 10 \mu\text{M}$ of various MT stabilizers and destabilizers was added before the addition of tubulin. In one instance, tubulin was pre-incubated on ice with colchicine for 15 min before addition to the cuvette. The stirrer setting on the instrument was maintained at 70%, while the temperature was set at 37°C .

3. Results and discussion

3.1. Tubulin polymerization on surfaces

Initial attempts to immobilize tubulin directly onto non-functionalized surfaces were unsuccessful, most likely due to denaturation of the protein upon contact with the somewhat hydrophobic biosensor surfaces (Andrade and Hlady, 1986). After trials with various functionalized surfaces, biotinylated surfaces appeared to provide the best environment for a streptavidin layer followed by subsequent biotinylated tubulin and MT layers. Streptavidin deposition on a biotinylated Amine AnaChipTM formed a layer with a mass of 3.34 ng/mm^2 , thickness of 5.94 nm and a density of 0.56 g/cm^3 , consistent with previously obtained values and the dimensions of streptavidin derived from X-ray crystallography (Swann et al., 2003). The addition of biotinylated tubulin caused mass and thickness to increase to 4.12 ng/mm^2 and 7.18 nm , respectively, accompanied by an increase in density to $0.57(5) \text{ g/cm}^3$, which indicates binding of biotinylated tubulin to the streptavidin surface (Fig. 1). The increase in thickness above the streptavidin layer was only 1.24 nm , rather than the size of a tubulin heterodimer ($\sim 8 \text{ nm}$), accompanied by a slight increase of 0.01 g/cm^3 in the density. The small increase in mass (molar ratio of biotinylated tubulin to streptavidin of 1:7) suggested that the layer of tubulin formed may not be optically homogeneous, since it corresponds to a surface coverage by biotinylated tubulin of the order of 12%, compared to 35% coverage for streptavidin (Swann et al., 2004). The initial binding of biotinylated-tubulin to the streptavidin was rapid, however, which suggested the association of biotinylated-tubulin to streptavidin to be specific, while the increase in density indicated that other processes occurred simultaneously. Thus, an alternative, but not mutually exclusive explanation is that, since tubulin has many exposed lysine residues, biotin may occupy multiple positions on the heterodimer and at various locations throughout, resulting in a number of different orientations of biotinylated-tubulin bound to streptavidin. For example, heterodimers that lie on their sides might fill any gaps between streptavidin molecules, accounting for the small thickness and density increase. Other orientations are likely to either promote or block subsequent binding of heterodimers to the surface depending on its surrounding environment. Nonetheless, the immobilized biotinylated-tubulin was sufficient to provide a starting point for MT growth. Any unbound biotinylated-tubulin was then washed away when the injection was complete and flow returned to running buffer. The subsequent introduction of tubulin in sample buffer caused a significant increase in mass and thickness reaching up to 22.85 nm with a continuous decrease in density to 0.40 g/cm^3 . The increase in thickness provided evidence that MTs were growing vertically on the surface, while the decrease in density is in accord with the formation of hollow MTs that are quite sparse, rather than continuous layering of tubulin dimers onto the surface. The first 10 min of the process may represent the initial nucleation and elongation process of protofilaments at a rate of 0.236 nm/min before an inflection point, where the extended

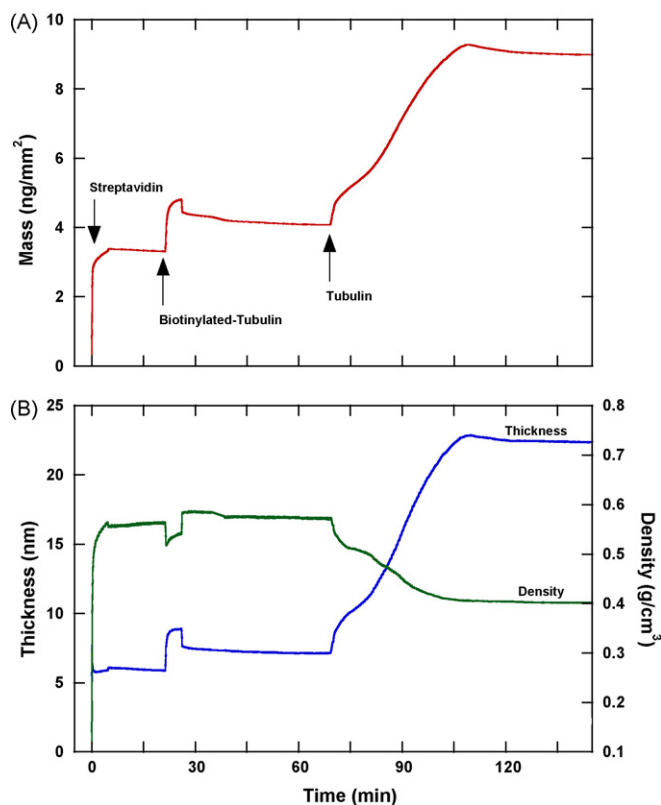


Fig. 1. Resolved mass, thickness, and density of tubulin assembly as assessed with DPI. (A) The increase in mass when streptavidin, biotinylated-tubulin, and tubulin were added indicated specific binding to each of the underlying layers. (B) The increase in thickness with decreasing density after the addition of tubulin to biotinylated tubulin suggested the formation of MTs on the surface.

protofilaments join together laterally to form the microtubule and the growth process accelerated to 0.436 nm/min (Fig. 2). Microtubule growth was halted in instances where the tubulin flow was decreased or stopped for an incubation, which reflects the need for mixing to take place near the surface. In a flow system, which is limited by the properties of laminar flow, mixing can only occur by flowing the sample relatively rapidly. We also attempted to clean DPI chips for re-use with various surfactant cleaners, sodium dodecyl sulfate, ethanol/acetone, etc., all while sonicating the chips followed by rinsing with deionized water. When chips were placed back into the instrument, however, the fringes had very low contrast and baseline signal was not stable enough to perform an experiment. Other harsher methods that etch the surface could be used, but may require the reintroduction of amine functional groups onto the chip surface before use.

Once MT polymerization was established on such surfaces using data from DPI, the assembly of MTs was evaluated using the RM cuvette system in an effort to establish a technique that conserves sample, is more user-friendly and applicable to larger sampling sets. The addition of soluble tubulin to a biotinylated-tubulin-streptavidin RM surface containing sample buffer at 37°C caused a bulk shift over the first 5 s, followed by a continuous increase in response (Fig. 3), corresponding to an increase in mass at the surface. In other attempts to assemble MTs on the surface, the addition of GTP after tubulin caused the increase in response to halt, possibly due to the polymerization of tubulin in solution rather than on the surface. We envision that the presence of a high concentration of GTP near the surface of the cuvette and the biotinylated tubulin allows for the tubulin to diffuse towards and bind to the surface. There were also occasional "bursts" of sudden

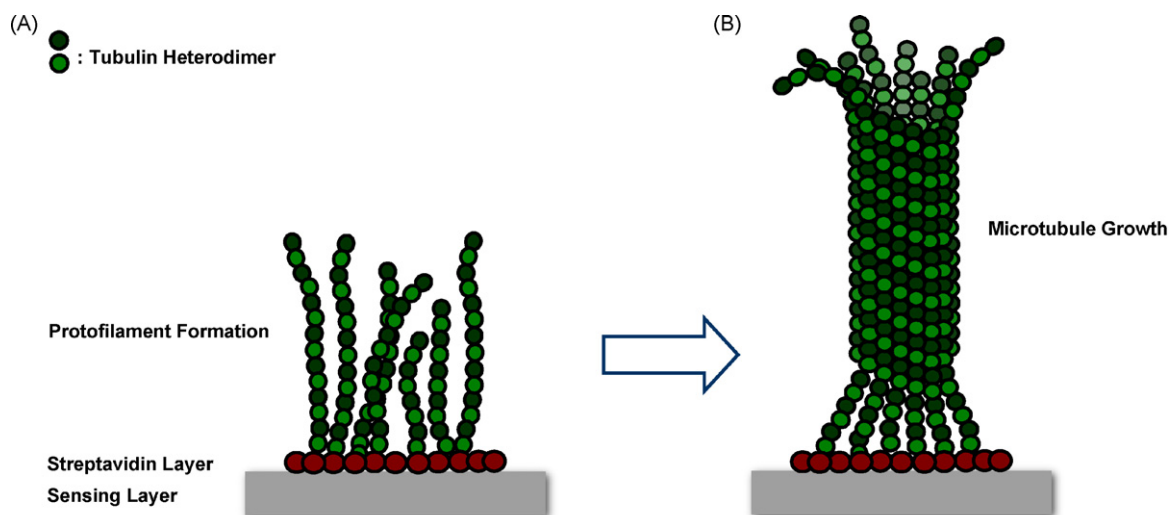


Fig. 2. (A) Schematic representation of the formation of a uniform streptavidin layer on a biotin chip followed by the growth of tubulin protofilaments on biotinylated tubulin. (B) As protofilaments continue to grow in length, they join laterally to form MTs.

increase in response that were observed (Fig. 3), suggesting periods of increased assembly and disassembly events. The return of response back to baseline after a buffer wash indicates the removal of the polymerized tubulin layer rather than the underlying biotinylated tubulin layer. Tubulin polymerization on such surfaces was reproducible over several (~20) cycles, but activity was lost once the surface was left at room temperature overnight or at 4 °C.

3.2. Temperature dependent polymerization

A key feature of tubulin polymerization is its temperature dependence (Hamel, 2003), with the ordered assembly of mammalian tubulin occurring optimally at 37 °C, while cold temperatures tend to destabilize MTs. Turbidimetry, most commonly used to measure MT polymerization and the influence of test agents on tubulin/MT dynamics (Hamel, 2003; Hall and Minton, 2005), is typically started at 0–4 °C and the change in absorbance is recorded while increasing the temperature to ≥ 26 °C (typically 30 °C or 37 °C) and subsequently dropping the temperature back to 4 °C to determine the stability of the MTs. The temperature dependence of tubulin polymerization was tested on the RM surface to demonstrate the functionality of tubulin on biosensor surfaces. Due to fogging of the optical system, the lowest temperature achievable

on the system was 15 °C, which is still an acceptable starting point for soluble (unpolymerized) tubulin, since normal polymerization does not take place at temperatures below 15 °C (Olmsted and Borisy, 1973). Since refractive index is also temperature-dependent (Grassi and Georgiadis, 1999), the response from a separate channel (reference channel) containing only sample buffer was subtracted from the sample channel to obtain the absolute change in response upon temperature change. The binding of tubulin to the surface at 15 °C was a slow process (Fig. 4) and reached a plateau with a lower response rather than exhibiting the continuously increasing response observed at 37 °C (Fig. 3). The lower response of tubulin binding at 15 °C was most likely due to the lower affinity of heterodimer association at such temperatures. Once temperature was increased to 37 °C, the absolute change in response increased, suggesting increased binding to the surface. It is worth mentioning that the change in response (Fig. 4) does not correspond to the observations from (Fig. 3) most likely due to the preference of tubulin to polymerize in solution rather than on the surface with

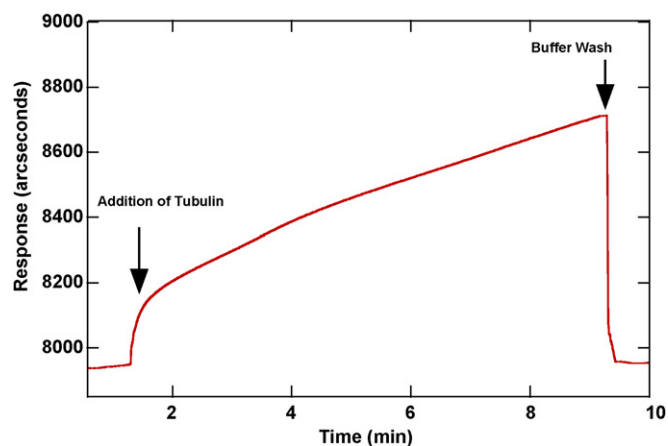


Fig. 3. Binding profile of tubulin to a biotinylated tubulin surface in 1 M MSG buffer (pH 6.6) with GTP at 37 °C using the RM biosensor. The response continuously increases until a buffer wash is performed and the process repeated.

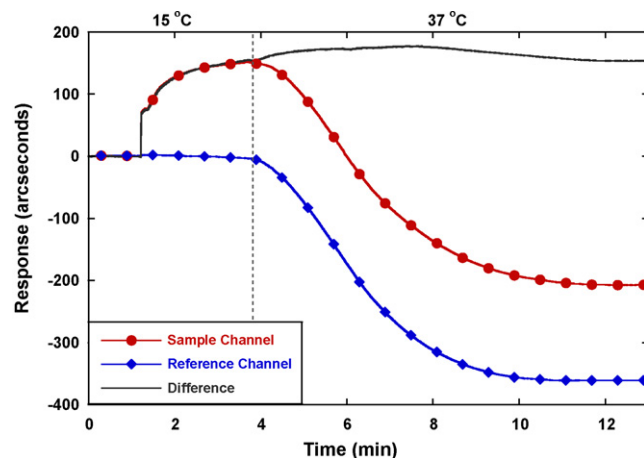


Fig. 4. Temperature dependence of tubulin binding to biotinylated tubulin using the RM biosensor. "Sample Channel" represents the observed response of the instrument upon the addition of tubulin to a biotinylated tubulin surface at 15 °C followed by an increase in temperature to 37 °C, where the dotted line indicates the start of temperature change. "Reference Channel" represents the change in response due to the change in temperature. "Difference" represents the subtraction of "Reference Channel" from the "Sample Channel" to give the absolute change in response due to the change in temperature.

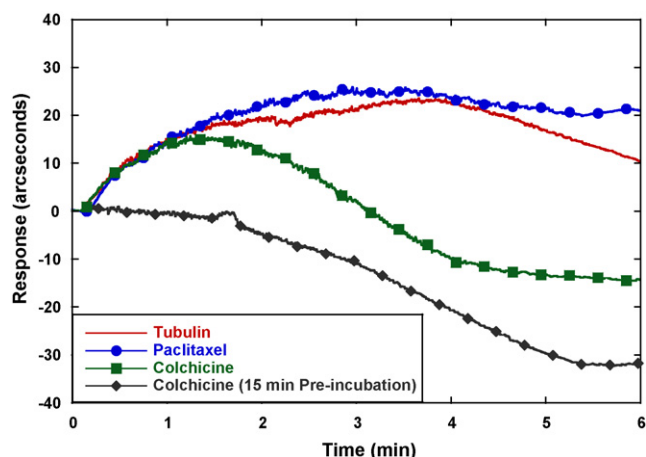


Fig. 5. The absolute change in response starting from the time of temperature change from 15 to 37 °C. Paclitaxel addition gave a similar response to tubulin with GTP, but remained more stable from MT disassembly afterwards. Colchicine caused a decrease in the response, after an initial increase in response, but completely inhibited binding when it was pre-incubated with tubulin.

such conditions. MT polymerization in solution most likely occurs in both situations due to the constant collisions of tubulin heterodimers and protofilaments with each other. We speculate the difference in the amount of solution polymerization is a result of Fick's law of diffusion; at lower temperatures, mass transfer of the macromolecules to the surface is decreased due to the increased viscosity of the solution (Seeton, 2006). As a result, there is less protofilament seeding occurring near the surface at 15 °C and polymerization in solution becomes more favorable once temperature is increased. When tubulin is introduced at 37 °C, mass transfer of the tubulin to the surface is greater and the current temperature allows for binding to biotinylated tubulin and further MT polymerization onto the surface. Previous SPR studies have also indicated that binding association constants of antigen–antibody interactions are greater when temperature is increased (Zeder-Lutz et al., 1997), supporting the argument that increased mass transfer at higher temperatures affects the rate of interactions at a surface.

In an effort to evaluate the effect of stabilizers and destabilizers on the binding of tubulin to biotinylated-tubulin surfaces, either paclitaxel or colchicine, respectively were introduced to the mixture. When paclitaxel was added to the cuvette before the addition of tubulin, a similar binding profile was observed, even after the temperature change (Fig. 5). The binding response was also generally more stable to subsequent decreases in absolute response observed after a few minutes with tubulin alone, which most likely represent events of MT disassembly. Colchicine had a delayed inhibition effect when added before tubulin, in all likelihood due to its slow association rate with tubulin (Banerjee et al., 1997), since it completely inhibited binding when pre-incubated with tubulin on ice for 15 min prior to the addition of the tubulin to the cuvette. This provides further evidence that microtubules bound to the surface are functional and exhibit similar characteristics as those in solution.

3.3. Comparison of MT-perturbing compounds

The binding profile of microtubule polymerization generally did not fit well to mono- or bi-phasic binding profiles upon analysis with Fastfit Software (NeoSensors, Ltd., UK). This is not surprising, given the complexity of MT polymerization (Hall and Minton, 2005), which involves many processes, such as GTP binding and hydrolysis, as well as constant assembly and disassembly events

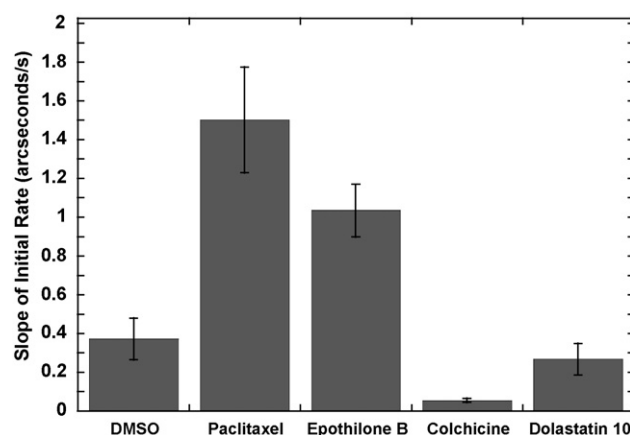


Fig. 6. Comparison of the slopes of initial rates of MT assembly with various MT-perturbing agents. Paclitaxel and epothilone B, both MT stabilizers, exhibited greater slopes of initial rates in comparison with the DMSO control. MT destabilizers, colchicine and dolastatin, gave lower values than the control. Values are reported as a mean with $n=3$ and the error bars representing the standard deviation. The pair-wise P -values in comparison with the tubulin control were 0.035, 0.005, 0.033, and 0.838 for paclitaxel, epothilone B, colchicine, and dolastatin 10, respectively.

(Jordan and Wilson, 2004). The most consistent parameter that describes interactions of tubulin with various agents appears to be the slopes of initial rates of assembly; the change in response during the first 15 s after tubulin is added (allowing for a 5 s mixing time during the bulk shift). These values appear to provide a means to evaluate the polymerization process in the presence of various de-/stabilizer agents. When paclitaxel and epothilone B, both MT stabilizers, were introduced to the system, the slopes of initial rates (mean $\pm$ SD, $n=3$) were significantly higher at 1.50 ± 0.27 and 1.04 ± 0.13 arcsconds/s, respectively, compared to 0.37 ± 0.11 arcsconds/s for tubulin containing GTP only (Fig. 6). This is expected given tubulin in the presence of paclitaxel polymerizes at a faster rate than in the presence of epothilone B (Gertsch et al., 2009). In the presence of MT destabilizers such as colchicine and dolastatin 10, the slopes of initial rates were lower than in their absence at 0.05 ± 0.01 and 0.27 ± 0.08 arcsconds/s, respectively. Thus, the present approach provides a means for a screening, differentiating compounds acting as stabilizers from those that are destabilizers based on the slopes of initial rates.

4. Conclusions

Characterizing the effects of MT-perturbing agents on the dynamics and stability of MTs is an important step to identify effective anti-cancer drugs. The detailed information of mass, thickness, and density of protein layers provided by DPI indicated the formation of MTs after an initial nucleation process over a streptavidin-biotinylated tubulin surface. The formation of MTs was also evaluated by RM using a cuvette-based system that consumes less protein sample and is more applicable for sampling larger sets of compounds. The temperature dependence of tubulin assembly with various MT de-/stabilizers agreed well with previously obtained turbidimetric results. Finally, the initial rates of tubulin assembly with MT de-/stabilizers provided a possible parameter to distinguish MT stabilizers from destabilizers. We envision future work to include, in addition to the measurement of initial rates using optical biosensors, the formation of MTs on surfaces of microtiter plates based on the methods described above and applying other types of high-throughput screening techniques such as fluorescence arrays to screen libraries of MT-perturbing agents.

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References

- Andrade, J.D., Hlady, V., 1986. *Advances in Polymer Science* 79, 1–63.
- Aoyama, H., Noguchi, T., Misawa, T., Nakamura, T., Miyachi, H., Hashimoto, Y., Kobayashi, H., 2007. *Chemical & Pharmaceutical Bulletin* 55, 944–949.
- Banerjee, S., Chakrabarti, G., Bhattacharyya, B., 1997. *Biochemistry* 36, 5600–5606.
- Bhattacharyya, B., Panda, D., Gupta, S., Banerjee, M., 2008. *Medicinal Research Reviews* 28 (1), 155–183.
- Boozer, C., Kim, G., Cong, S., Guan, H., Londergan, T., 2006. *Current Opinion in Biotechnology* 17 (4), 400–405.
- Burns, R.G., 1991. *Cell Motility and the Cytoskeleton* 20 (3), 181–189.
- Cross, G.H., Reeves, A., Brand, S., Swann, M.J., Peel, L.L., Freeman, N.J., Lu, J.R., 2004. *Journal of Physics D: Applied Physics* 37, 74–80.
- Cush, R., Cronin, J.M., Stewart, W.J., Maule, C.H., Molloy, J., Goddard, N.J., 1993. *Biosensors and Bioelectronics* 8, 347–353.
- Drukman, S., Kavallaris, M., 2002. *International Journal of Oncology* 21, 621–628.
- Gertsch, J., Meier, S., Müller, M., Altmann, K.H., 2009. *ChemBioChem* 10 (1), 166–175.
- Grassi, J.H., Georgiadis, R.M., 1999. *Analytical Chemistry* 71 (19), 4392–4396.
- Hall, D., Minton, A.P., 2005. *Analytical Biochemistry* 345 (2), 198–213.
- Hamel, E., Lin, C.M., 1984. *Biochemistry* 23, 4173–4184.
- Hamel, E., 1992. *Pharmacology and Therapeutics* 55, 31–51.
- Hamel, E., 2003. *Cell Biochemistry and Biophysics* 38, 1–21.
- Homola, J., 2003. *Analytical and Bioanalytical Chemistry* 377, 528–539.
- Howard, W.D., Timasheff, S.N., 1998. *Journal of Biological Chemistry* 263 (3), 1342–1346.
- Jordan, M.A., Wilson, L., 1998. *Current Opinion in Cell Biology* 10, 123–130.
- Jordan, M.J., Wilson, L., 2004. *Nature Reviews: Cancer* 4, 253–265.
- Karlsson, R., 2004. *Journal of Molecular Recognition* 17 (3), 151–161.
- Kumar, N., 1981. *Journal of Biological Chemistry* 256 (20), 10435–10441.
- Lillis, B., Manning, M., Berney, H., Hurley, E., Mathewson, A., Sheehan, M.M., 2006. *Biosensors and Bioelectronics* 21 (8), 1459–1467.
- Lukosz, W., 1991. *Biosensors and Bioelectronics* 6, 215–225.
- MacNeal, R.K., Purich, D.L., 1978. *Journal of Biological Chemistry* 253, 4683–4687.
- Nellen, P.M., Lukosz, W., 1991. *Biosensors and Bioelectronics* 6, 215–225.
- Olmsted, J.B., Borisy, G.G., 1973. *Biochemistry* 12 (21), 4282–4289.
- Popplewell, J.F., Swann, M.J., Ahmed, Y., Turnbull, J.E., Fernig, D.G., 2009. *Chem-BioChem* 10 (7), 1218–1226.
- Sackett, D.L., 1995. *Biochemistry* 34 (21), 7010–7019.
- Saxton, W.M., Stemple, D.L., Leslie, R.J., Salmon, E.D., Zavortink, M., McIntosh, J.R., 1984. *Journal of Cell Biology* 99, 2175–2186.
- Seeton, C.J., 2006. *Tribology Letters* 22 (1), 67–78.
- Swann, M., Freeman, N., Carrington, S., Ronan, G., Barrett, P., 2003. *Letters in Peptide Science* 10, 487–494.
- Swann, M.J., Peel, L.L., Carrington, S., Freeman, N.J., 2004. *Analytical Biochemistry* 329, 190–198.
- Wade, R.H., Chretien, D., 1993. *Journal of Structural Biology* 110, 1–27.
- Zeder-Lutz, G., Zuber, E., Witz, J., Van Regenmortel, M.H.V., 1997. *Analytical Biochemistry* 246, 123–132.